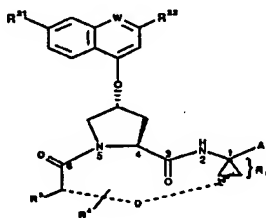




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C07K 5/08, 5/078, A61K 38/05, 38/06, A61P 31/14	A1	(11) International Publication Number: WO 00/59929 (43) International Publication Date: 12 October 2000 (12.10.00)
(21) International Application Number: PCT/CA00/00353 (22) International Filing Date: 3 April 2000 (03.04.00) (30) Priority Data: 60/128,011 6 April 1999 (06.04.99) US (71) Applicant (for all designated States except US): BOEHRINGER INGELHEIM (CANADA) LTD. [CA/CA]; 2100 Cunard Street, Laval, Québec H7S 2G5 (CA). (72) Inventors; and (75) Inventors/Applicants (for US only): TSANTRIZOS, Youla, S. [CA/CA]; 1590 Champigny, Saint-Laurent, Québec H4L 4P7 (CA). CAMERON, Dale, R. [CA/CA]; 493 de L'Erablière, Rosemère, Québec J7A 4M4 (CA). FAUCHER, Anne-Marie [CA/CA]; 11 Lefebvre Nord, Oka, Québec JON 1E0 (CA). GHIRO, Elise [CA/CA]; 768 Pierre, Laval, Québec H7X 3L8 (CA). GOUDREAU, Nathalie [CA/CA]; 416 Graham, Mont-Royal, Québec H3P 2C9 (CA). HALMOS, Teddy [CA/CA]; 1935 Jean Picard #8, Laval, Québec H7T 2K4 (CA). LLINAS-BRUNET, Montse [CA/CA]; 14 Rushbrooke, Dollard-Des-Ormeaux, Québec H9B 3K6 (CA).	(74) Agent: BERNIER, Louise, G.; Boehringer Ingelheim (Canada) Ltd., 2100 Cunard, Laval, Québec H7S 2G5 (CA). (81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>	

(54) Title: MACROCYCLIC PEPTIDES ACTIVE AGAINST THE HEPATITIS C VIRUS



(1)

(57) Abstract

The present invention covers macrocyclic compounds of formula (I) active in-vitro and in cellular assays against the NS3 protease of the hepatitis C virus wherein W is CH or N; R²¹ is H, halo, C₁₋₆ alkyl, cycloalkyl, haloalkyl, C₁₋₆ alkoxy, cycloalkoxy, hydroxy, or N(R²³)₂, wherein each R²³ is independently H, C₁₋₆ alkyl or cycloalkoxy; and R²² is H, halo, C₁₋₆ alkyl, C₃₋₆ cycloalkyl, C₁₋₆ haloalkyl, C₁₋₆ thioalkyl, C₁₋₆ alkoxy, C₃₋₆ cycloalkoxy, C₂₋₇ alkoxyalkyl, C₃₋₆ cycloalkyl, C₆ or 10 aryl or Het, wherein Het is a five-, six-, or seven-membered saturated or unsaturated heterocycle containing from one to four heteroatoms selected from nitrogen, oxygen and sulfur; said cycloalkyl, aryl or Het being substituted with R²⁴, wherein R²⁴ is H, C₁₋₆ alkyl, C₃₋₆ cycloalkyl, C₁₋₆ alkoxy, C₃₋₆ cycloalkoxy, NO₂, N(R²⁵)₂, NH-C(O)-R²⁵, or NH-C(O)-NH-R²⁵, wherein each R²⁵ is independently: H, C₁₋₆ alkyl or C₃₋₆ cycloalkyl; or R²⁴ is NH-C(O)-OR²⁶ wherein R²⁶ is C₁₋₆ alkyl or C₃₋₆ cycloalkyl; R³ is hydroxy, NH₂, or a group of formula -NH-R³¹, wherein R³¹ is C₆ or 10 aryl, heteroaryl, -C(O)-R³², -C(O)-OR³² or -C(O)-NHR³², wherein R³² is: C₁₋₆ alkyl or C₃₋₆ cycloalkyl; D is a 5 to 10-atom saturated or unsaturated alkylene chain optionally containing one to three heteroatoms independently selected from: O, S, or N-R⁴¹, wherein R⁴¹ is H, C₁₋₆ alkyl, cycloalkyl or -C(O)-R⁴², wherein R⁴² is C₁₋₆ alkyl, cycloalkyl or C₆ or 10 aryl; R⁴ is H or from one to three substituents at any carbon atom of said chain D, said substituent independently selected from the group consisting of: C₁₋₆ alkyl, C₁₋₆ haloalkyl, C₁₋₆ alkoxy, hydroxy, halo, amino, oxo, thio or C₁₋₆ thioalkyl, and A is an amide of formula -C(O)-NH-R⁵, wherein R⁵ is selected from the group consisting of: C₁₋₈ alkyl, C₃₋₆ cycloalkyl, C₆ or 10 aryl or C₇₋₁₆ aralkyl; or A is a carboxylic acid or a pharmaceutically acceptable salt or ester thereof.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

MACROCYCLIC PEPTIDES ACTIVE AGAINST THE HEPATITIS C VIRUS

FIELD OF THE INVENTION

The present invention relates to compounds, compositions, the preparation of such
5 compounds and methods for the treatment of hepatitis C virus (HCV) infection. In
particular, the present invention provides novel peptide analogues, pharmaceutical
compositions containing such analogues and methods for using these analogues in
the treatment of HCV infection.

BACKGROUND OF THE INVENTION

10 Hepatitis C virus (HCV) is the major etiological agent of post-transfusion and
community-acquired non-A non-B hepatitis worldwide. It is estimated that over 170
million people worldwide are infected by the virus. A high percentage of carriers
become chronically infected and many progress to chronic liver disease, so-called
chronic hepatitis C. This group is in turn at high risk for serious liver disease such
15 as liver cirrhosis, hepatocellular carcinoma and terminal liver disease leading to
death.

The mechanism by which HCV establishes viral persistence and causes a high rate of
chronic liver disease has not been thoroughly elucidated. It is not known how HCV
interacts with and evades the host immune system. In addition, the roles of cellular
20 and humoral immune responses in protection against HCV infection and disease
have yet to be established. Immunoglobulins have been reported for prophylaxis of
transfusion-associated viral hepatitis, however, the Center for Disease Control does
not presently recommend immunoglobulins treatment for this purpose. The lack of
an effective protective immune response is hampering the development of a vaccine
25 or adequate post-exposure prophylaxis measures, so in the near-term, hopes are
firmly pinned on antiviral interventions.

Various clinical studies have been conducted with the goal of identifying
pharmaceutical agents capable of effectively treating HCV infection in patients
afflicted with chronic hepatitis C. These studies have involved the use of interferon-
30 alpha, alone and in combination with other antiviral agents. Such studies have
shown that a substantial number of the participants do not respond to these
therapies, and of those that do respond favorably, a large proportion were found to

relapse after termination of treatment.

Until a few years ago, interferon (IFN) was the only available therapy of proven benefit approved in the clinic for patients with chronic hepatitis C. However the sustained response rate is low, and interferon treatment also induces severe side-effects (i.e. retinopathy, thyroiditis, acute pancreatitis, depression) that diminish the quality of life of treated patients. Interferon in combination with ribavirin was originally approved for patients non-responsive to IFN alone. It has now been approved for naïve patients and presently constitutes the gold standard in HCV therapy. However, the side effects caused by IFN are not alleviated with this combination therapy.

Therefore, a need exists for the development of effective antiviral agents for treatment of HCV infection that overcomes the limitations of existing pharmaceutical therapies.

HCV is an enveloped positive strand RNA virus in the Flaviviridae family. The single strand HCV RNA genome is approximately 9500 nucleotides in length and has a single open reading frame (ORF) encoding a single large polyprotein of about 3000 amino acids. In infected cells, this polyprotein is cleaved at multiple sites by cellular and viral proteases to produce the structural and non-structural (NS) proteins. In the case of HCV, the generation of mature nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) is effected by two viral proteases. The first one, as yet poorly characterized, cleaves at the NS2-NS3 junction; the second one is a serine protease contained within the N-terminal region of NS3 (henceforth referred to as NS3 protease) and mediates all the subsequent cleavages downstream of NS3, both in *cis*, at the NS3-NS4A cleavage site, and in *trans*, for the remaining NS4A-NS4B, NS4B-NS5A, NS5A-NS5B sites. The NS4A protein appears to serve multiple functions, acting as a cofactor for the NS3 protease and possibly assisting in the membrane localization of NS3 and other viral replicase components. The complex formation of the NS3 protein with NS4A seems necessary to the processing events, enhancing the proteolytic efficiency at all of the sites. The NS3 protein also exhibits nucleoside triphosphatase and RNA helicase activities. NS5B is a RNA-dependent RNA polymerase that is involved in the replication of HCV.

Patent application WO 97/06804 describes the (-) enantiomer of the nucleoside

3

analogue cytosine-1,3-oxathiolane (also known as 3TC) as active against HCV. This compound, although reported as safe in previous clinical trials against HIV and HBV, has yet to be clinically proven active against HCV and its mechanism of action against the virus has yet to be reported.

- 5 A general strategy for the development of antiviral agents is to inactivate virally encoded enzymes that are essential for the replication of the virus
- In this vein, intense efforts to discover compounds which inhibit the NS3 protease or RNA helicase of HCV have led to the following disclosures:

US patent 5,633,388 describes heterocyclic-substituted carboxamides and analogues
10 as being active against HCV. These compounds are directed against the helicase activity of the NS3 protein of the virus but clinical tests have not yet been reported. A phenanthrenequinone has been reported by Chu et al., (Tet. Lett., (1996), 7229-7232) to have activity against the HCV NS3 protease *in vitro*. No further development on this compound has been reported.

- 15 A paper presented at the Ninth International Conference on Antiviral Research, Urabandai, Fukuyshima, Japan (1996) (Antiviral Research, (1996), 30, 1, A23 (abstract 19)) reports thiazolidine derivatives to be inhibitory to the HCV protease.
- Several studies have reported compounds inhibitory to other serine proteases, such as human leukocyte elastase. One family of these compounds is reported in WO
20 95/33764 (Hoechst Marion Roussel, 1995). The peptides disclosed in that application are morpholinylcarbonyl-benzoyl-peptide analogues that are structurally different from the peptides of the present invention.

WO 98/17679 from Vertex Pharmaceuticals Inc. discloses inhibitors of serine protease, particularly, Hepatitis C virus NS3 protease

- 25 Hoffman LaRoche (WO 98/22496; US 5,866,684 & US 6,018,020) has also reported hexapeptides that are proteinase inhibitors useful as antiviral agents for the treatment of HCV infection.

Steinkühler *et al.* and Ingallinella *et al.* have published on NS4A-4B product inhibition (Biochemistry (1998), 37, 8899-8905 and 8906-8914).

- 30 WO 97/43310 by Schering Corporation discloses 20 and 21 amino acid peptide sequences active against the HCV NS3 protease.

WO 98/46597 by Emory University discloses peptides and peptidomimetics active

in vitro against serine proteases.

WO 98/46630 by Peptide Therapeutics Limited discloses depsipeptide substrate inhibiting the HCV NS3 protease.

Finally, US 5,869,253 discloses enzymatic RNA molecules that inhibit the HCV NS3 protease.

None of the prior patent applications described above disclose suggest cyclic peptides active and selective against the Hepatitis C virus NS3 protease.

WO 99/07733, WO 99/07734, WO 00/09543 and WO00/09558 disclose hexa to tetrapeptides and tripeptide analogs that inhibit the NS3 protease. However, these disclosures do not suggest or lead to the design of macrocyclic analogs of the present invention.

WO 99/38888 published August 5, 1999 by the Institute de Recherche di Biologia Moleculare (IRBM) discloses small peptides inhibitors of the HCV NS3 protease. Nothing in this disclosure suggest or indicates the cyclic nature of the peptides of the present invention. In addition, this PCT application was published after the priority date of the present application.

WO 99/64442 by IRBM, also published after the priority date of this application, discloses oligopeptides with ketoacids at P1.

WO 99/50230 from Vertex Pharmaceuticals (published on October 7, 1999) was also published after the priority date of the present application. Even then, the publication does not remotely suggest any cyclic peptides of the present invention.

One advantage of the present invention is that it provides macrocyclic peptides that are inhibitory to the NS3 protease of the hepatitis C virus.

A further advantage of one aspect of the present invention resides in the fact that these peptides specifically inhibit the NS3 protease and do not show significant inhibitory activity against other serine proteases such as human leukocyte elastase (HLE), porcine pancreatic elastase (PPE), or bovine pancreatic chymotrypsin, or cysteine proteases such as human liver cathepsin B (Cat B).

A further advantage of the present invention is that it provides small peptides of low molecular weight that are capable of penetrating cell membranes and inhibit the NS3 protease activity in cell culture.

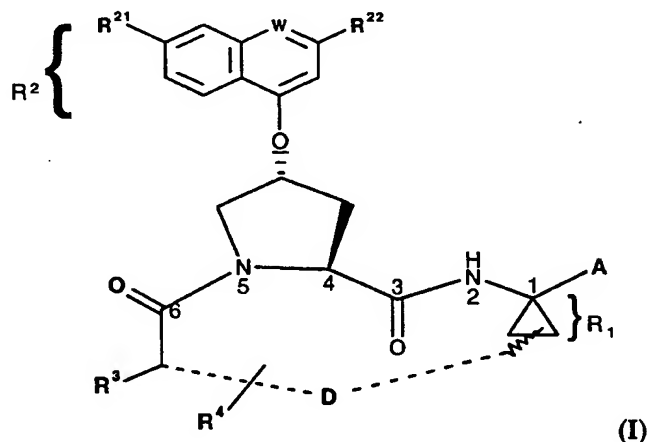
5

Still, a further advantage of the compounds of the present invention resides in the fact that they are active in both major genotypes found in clinical isolates (1a & 1b), strongly suggesting that these compound will be active against all presently known genotypes of HCV.

5

SUMMARY OF THE INVENTION

Included in the scope of the invention are compounds of formula (I):



wherein W is CH or N,

10 R^{21} is H, halo, C_{1-6} alkyl, C_{3-6} cycloalkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{3-6} cycloalkoxy, hydroxy, or $N(R^{23})_2$, wherein each R^{23} is independently H, C_{1-6} alkyl or C_{3-6} cycloalkyl; and

R^{22} is H, halo, C_{1-6} alkyl, C_{3-6} cycloalkyl, C_{1-6} haloalkyl, C_{1-6} thioalkyl, C_{1-6} alkoxy, C_{3-6} cycloalkoxy, C_{2-7} alkoxyalkyl, C_{3-6} cycloalkyl, $C_{6 \text{ or } 10}$ aryl or Het, wherein Het is a

15 five-, six-, or seven-membered, saturated or unsaturated heterocycle, containing from one to four heteroatoms selected from nitrogen, oxygen and sulfur; said cycloalkyl, aryl or Het being substituted with R^{24} ,

wherein R^{24} is H, halo, C_{1-6} alkyl, C_{3-6} cycloalkyl, C_{1-6} alkoxy, C_{3-6} cycloalkoxy, NO_2 , $N(R^{25})_2$, $NH-C(O)-R^{25}$, or $NH-C(O)-NH-R^{25}$,

20 wherein each R^{25} is independently: H, C_{1-6} alkyl or C_{3-6} cycloalkyl;

or R^{24} is $NH-C(O)-OR^{26}$ wherein R^{26} is C_{1-6} alkyl or C_{3-6} cycloalkyl;

R^3 is hydroxy, NH_2 , or a group of formula $-NH-R^{31}$, wherein R^{31} is $C_{6 \text{ or } 10}$ aryl, heteroaryl, $-C(O)-R^{32}$, $-C(O)-OR^{32}$, or $-C(O)-NHR^{32}$,

wherein R^{32} is: C_{1-6} alkyl or C_{3-6} cycloalkyl;

D is a 5 to 10-atom saturated or unsaturated alkylene chain optionally containing
5 one to three heteroatoms independently selected from: O, S, or $N-R^{41}$, wherein
 R^{41} is H, C_{1-6} alkyl, C_{3-6} cycloalkyl, or $-C(O)-R^{42}$, wherein R^{42} is C_{1-6} alkyl, C_{3-6}
cycloalkyl or $C_{6 \text{ or } 10}$ aryl;

R^4 is H or from one to three substituents at any carbon atom of said chain D, said
substituent independently selected from the group consisting of: C_{1-6} alkyl,
10 C_{1-6} haloalkyl, C_{1-6} alkoxy, hydroxy, halo, amino, oxo, thio, or C_{1-6} thioalkyl
and

A is an amide of formula $-C(O)-NH-R^5$, wherein R^5 is selected from the group
consisting of: C_{1-8} alkyl, C_{3-6} cycloalkyl, $C_{6 \text{ or } 10}$ aryl or C_{7-16} aralkyl;
or A is a carboxylic acid or a pharmaceutically acceptable salt or ester thereof.

15

Included within the scope of this invention is a pharmaceutical composition
comprising an anti-hepatitis C virally effective amount of a compound of formula I,
or a therapeutically acceptable salt or ester thereof, in admixture with a
pharmaceutically acceptable carrier medium or auxiliary agent.

20

An important aspect of the invention involves a method of treating a hepatitis C
viral infection in a mammal by administering to the mammal an anti-hepatitis C
virally effective amount of the compound of formula I, or a therapeutically
acceptable salt or ester thereof or a composition as described above.

25

Another important aspect involves a method of inhibiting the replication of hepatitis
C virus by exposing the virus to a hepatitis C NS3 protease-inhibiting amount of the
compound of formula I, or a therapeutically acceptable salt or ester thereof or a
composition as described above.

Still another aspect involves a method of treating a hepatitis C viral infection in a
mammal by administering thereto an anti-hepatitis C virally effective amount of a

combination of the compound of formula I, or a therapeutically acceptable salt or ester thereof. According to one embodiment, the pharmaceutical compositions of this invention comprise an additional immunomodulatory agent. Examples of additional immunomodulatory agents include but are not limited to, α -, β -, and δ -interferons.

According to an alternate embodiment, the pharmaceutical compositions of this invention may additionally comprise an antiviral agent. Examples of antiviral agents include, ribavirin and amantadine.

According to another alternate embodiment, the pharmaceutical compositions of this invention may additionally comprise other inhibitors of HCV protease.

According to yet another alternate embodiment, the pharmaceutical compositions of this invention may additionally comprise an inhibitor of other targets in the HCV life cycle, such as helicase, polymerase, metalloprotease or IRES.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

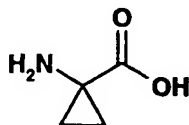
Definitions

As used herein, the following definitions apply unless otherwise noted:

With reference to the instances where (R) or (S) is used to designate the absolute configuration of a substituent, e.g. R⁴ of the compound of formula I, the designation is done in the context of the whole compound and not in the context of the substituent alone.

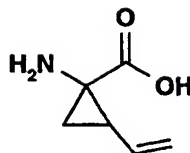
The designation "P1, P2, and P" as used herein refer to the position of the amino acid residues starting from the C-terminus end of the peptide analogs and extending towards the N-terminus (i.e. P1 refers to position 1 from the C-terminus, P2: second position from the C-terminus, etc.) (see Berger A. & Schechter I., Transactions of the Royal Society London series B257, 249-264 (1970)).

As used herein the term "1-aminocyclopropyl-carboxylic acid" (ACCA) refers to a compound of formula:

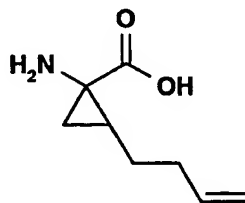


As used herein the term "vinyl-ACCA" refers to a compound of formula:

8



As used herein the term "homo-allyl-ACCA" refers to a compound of formula:



The term "halo" as used herein means a halogen substituent selected from bromo,
5 chloro, fluoro or iodo.

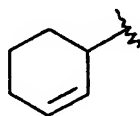
The term " C_{1-6} haloalkyl" as used herein means as used herein, either alone or in combination with another substituent, means acyclic, straight or branched chain alkyl substituents containing from 1 to six carbon atoms having one or more hydrogen substituted for a halogen selected from bromo, chloro, fluoro or iodo.

10 The term " C_{1-6} thioalkyl" as used herein means as used herein, either alone or in combination with another substituent, means acyclic, straight or branched chain alkyl substituents containing a thiol group such a thiopropyl.

The term " C_{1-6} alkyl" or "(lower)alkyl" as used herein, either alone or in combination with another substituent, means acyclic, straight or branched chain alkyl
15 substituents containing from 1 to six carbon atoms and includes, for example, methyl, ethyl, propyl, butyl, hexyl, 1-methylethyl, 1-methylpropyl, 2-methylpropyl, 1,1-dimethylethyl.

The term " C_{3-6} cycloalkyl" as used herein, either alone or in combination with another substituent, means a cycloalkyl substituent containing from three to six
20 carbon atoms and includes cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl.

The term "unsaturated cycloalkyl" includes, for example, the substituent cyclohexenyl:



The term "saturated or unsaturated alkylene" as used herein means a divalent alkyl substituent derived by the removal of one hydrogen atom from each end of a saturated or unsaturated straight or branched chain aliphatic hydrocarbon and includes, for example,

- 5 $-\text{CH}_2\text{CH}_2\text{C}(\text{CH}_3)_2\text{CH}_2\text{CH}_2-$, $-\text{CH}_2\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}_2-$ or $-\text{CH}_2\text{C}\equiv\text{CCH}_2\text{CH}_2-$. This alkyl chain may optionally contain a heteroatom such as oxygen (for example: $\text{CH}_3-\text{CH}_2-\text{O}-\text{CH}_2-$).

The term " C_{1-6} alkoxy" as used herein, either alone or in combination with another substituent, means the substituent $-\text{O}-\text{C}_{1-6}$ alkyl wherein alkyl is as defined above containing up to six carbon atoms. Alkoxy includes methoxy, ethoxy, propoxy, 1-methylethoxy, butoxy and 1,1-dimethylethoxy. The latter substituent is known commonly as *tert*-butoxy.

10

The term " C_{3-6} cycloalkyl" as used herein, either alone or in combination with another substituent, means the substituent $-\text{O}-\text{C}_{3-6}$ cycloalkyl containing from three to 6 carbon atoms.

15

The term " C_{1-6} alkoxyalkyl" as used herein, means the substituent C_{1-6} alkyl- $\text{O}-\text{C}_{1-6}$ alkyl wherein alkyl is as defined above containing up to six carbon atoms. For example, methoxymethyl means $-\text{CH}_2-\text{O}-\text{CH}_3$.

The term " C_{2-7} acyl" as used herein, either alone or in combination with another substituent, means an C_{1-6} alkyl group linked through a carbonyl group such as $-\text{C}(\text{O})-\text{C}_{1-6}$ alkyl.

20

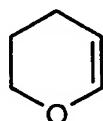
The term " C_6 or C_{10} aryl" as used herein, either alone or in combination with another substituent, means either an aromatic monocyclic system containing 6 carbon atoms or an aromatic bicyclic system containing 10 carbon atoms. For example, aryl includes a phenyl or a naphthyl – ring system.

25

The term " C_{7-16} aralkyl" as used herein, either alone or in combination with another substituent, means an aryl as defined above linked through an alkyl group, wherein alkyl is as defined above containing from 1 to 6 carbon atoms. Aralkyl includes for example benzyl, and butylphenyl.

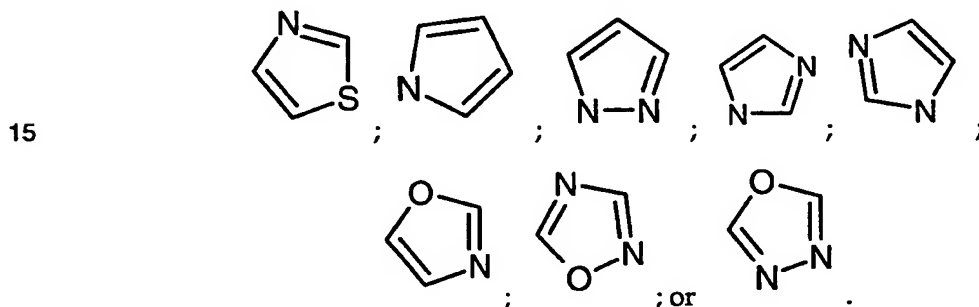
10

The term "Het" as used herein, either alone or in combination with another substituent, means a monovalent substituent derived by removal of a hydrogen from a five-, six-, or seven-membered saturated or unsaturated (including aromatic) heterocycle containing from one to four heteroatoms selected from nitrogen, oxygen and sulfur. Examples of suitable heterocycles include: tetrahydrofuran, thiophene, diazepine, isoxazole, piperidine, dioxane, morpholine, pyrimidine or

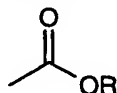


The term "Het" also includes a heterocycle as defined above fused to one or more other cycle be it a heterocycle or any other cycle. One such examples includes thiazolo[4,5-b]-pyridine.

Although generally covered under the term "Het", the term "heteroaryl" as used herein precisely defines an unsaturated heterocycle for which the double bonds form an aromatic system. Suitable example of heteroaromatic system include: quinoline, indole, pyridine,



The term "pharmaceutically acceptable ester" as used herein, either alone or in combination with another substituent, means esters of the compound of formula I in which any of the carboxyl functions of the molecule, but preferably the carboxy terminus, is replaced by an alkoxycarbonyl function:



in which the **R** moiety of the ester is selected from alkyl (e.g. methyl, ethyl, *n*-propyl, *t*-butyl, *n*-butyl); alkoxyalkyl (e.g. methoxymethyl); alkoxyacyl (e.g. acetoxymethyl); aralkyl (e.g. benzyl); aryloxyalkyl (e.g. phenoxyethyl); aryl (e.g. phenyl),

optionally substituted with halogen, C₁₋₄ alkyl or C₁₋₄ alkoxy. Other suitable prodrug esters are found in Design of prodrugs, Bundgaard, H. Ed. Elsevier (1985) incorporated herewith by reference. Such pharmaceutically acceptable esters are usually hydrolyzed *in vivo* when injected in a mammal and transformed into the acid form of the compound of formula I.

- With regard to the esters described above, unless otherwise specified, any alkyl moiety present advantageously contains 1 to 16 carbon atoms, particularly 1 to 6 carbon atoms. Any aryl moiety present in such esters advantageously comprises a phenyl group.
- 10 In particular the esters may be a C₁₋₁₆ alkyl ester, an unsubstituted benzyl ester or a benzyl ester substituted with at least one halogen, C₁₋₆ alkyl, C₁₋₆ alkoxy, nitro or trifluoromethyl.

The term "pharmaceutically acceptable salt" as used herein includes those derived from pharmaceutically acceptable bases. Examples of suitable bases include choline, ethanolamine and ethylenediamine. Na⁺, K⁺, and Ca⁺⁺ salts are also contemplated to be within the scope of the invention (also see Pharmaceutical salts, Birge, S.M. et al., J. Pharm. Sci., (1977), 66, 1-19, incorporated herein by reference).

Preferred embodiments

R¹:

- 20 Preferred embodiments of the present invention include compounds of formula I as described above, wherein the R¹ moiety is selected from the 2 different diastereoisomers where the 1-carbon center has the R configuration as represented by structures (i) and (ii):



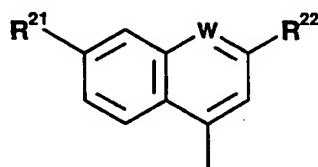
- 25 D syn to the amide (i), or D syn to A (ii).

More preferably, the linker D is linked to R¹ in the configuration *syn* to A as represented by structure (ii).

12

 R^2 :

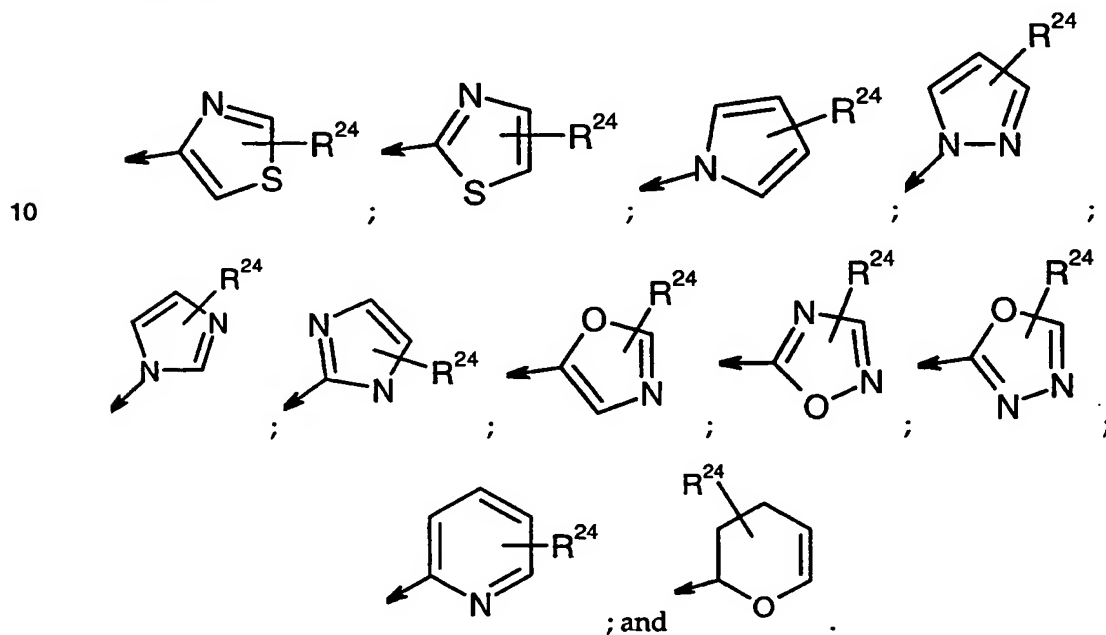
Preferred embodiments of the present invention include compounds of formula I as described above, wherein the R^2 moiety is



wherein W is preferably N.

- 5 Preferably, R^{21} is H, C_{1-6} alkyl, C_{1-6} alkoxy, hydroxy, chloro, or $N(R^{23})_2$ wherein R^{23} is preferably H or C_{1-6} alkyl. More preferably, R^{21} is H or C_{1-6} alkoxy. Most preferably, R^{21} is methoxy.

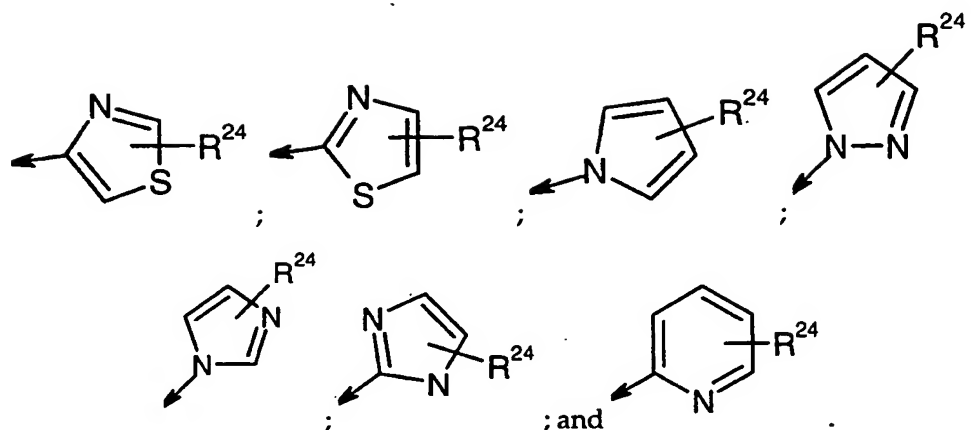
Preferably R^{22} is H, C_{1-6} thioalkyl, C_{1-6} alkoxy, phenyl or Het selected from the group consisting of:



Preferably, R^{24} is H, C_{1-6} alkyl, $NH-R^{25}$, $NH-C(O)-R^{25}$; or $NH-C(O)-NH-R^{25}$ or $NH-C(O)-OR^{26}$.

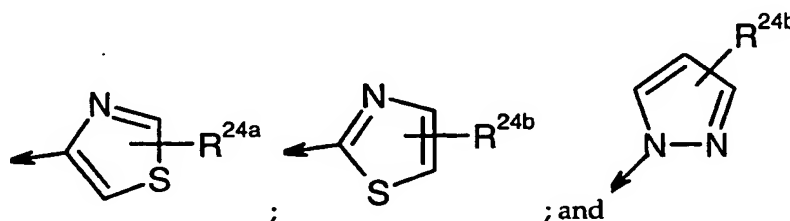
- 15 More preferably R^{22} is C_{1-4} alkoxy, phenyl or Het selected from the group consisting of:

13



More preferably, R^{24} is H, C_{1-6} alkyl, $NH-R^{25}$, $NH-C(O)-R^{25}$; or $NH-C(O)-OR^{26}$.

Most preferably R^{22} is ethoxy, or Het selected from the group consisting of:



5

Most preferably, R^{24a} is $NH-R^{25}$, $NH-C(O)-R^{25}$, or $NH-C(O)-OR^{26}$. Most preferably, R^{24b} is H or C_{1-6} alkyl.

Preferably, each R^{25} is independently: H, C_{1-6} alkyl, or C_{3-6} cycloalkyl. More preferably, R^{25} is C_{1-6} alkyl or C_{3-6} cycloalkyl. More preferably, R^{25} is C_{1-6} alkyl.

10 Preferably, R^{26} is C_{1-6} alkyl.

R^3 :

Preferred embodiments of the present invention include compounds of formula I as described above, wherein the R^3 moiety is preferably an amide of formula $NH-C(O)-$

15 R^{32} , a urea of formula $NH-C(O)-NH-R^{32}$, or a carbamate of formula $NH-C(O)-OR^{32}$.

More preferably, R^3 is a carbamate or a urea. Most preferably, R^3 is a carbamate.

Preferably, R^{32} is C_{1-6} alkyl, or C_{3-6} cycloalkyl. More preferably, R^{32} is C_{1-6} alkyl, or C_{4-6} cycloalkyl. Most preferably, R^{32} is *tert*-butyl, cyclobutyl or cyclopentyl.

D:

Preferred embodiments of the present invention include compounds of formula I, wherein linker D is a 6 to 8 atom saturated or unsaturated alkylene chain. More preferably, linker D is 7 atom chain.

- 5 Preferably, the D chain contains one or two heteroatoms selected from: O, S, NH, N-C₁₋₆ alkyl or N-C₂₋₇ acyl. More preferably, the D chain optionally contains one heteroatom selected from: NH, or N-C₂₋₇ acyl, most preferably N(Ac), and is positioned at atom 10 of the chain. Most preferably, the chain containing a nitrogen atom is saturated.
- 10 Alternatively, D contains one heteroatom selected from: O, or S. Preferably, when D is 7 atom in length, the O or S atom is at position 9 of the chain. Preferably, this chain is substituted with R⁴, wherein R⁴ is H or C₁₋₆ alkyl. More preferably, R⁴ is H or methyl. Most preferably, R⁴ is H or 8-(S)-Me. Even most preferably, D is saturated. Alternatively, D contains one double bond at position 11,12. Preferably, this double
- 15 bond is *trans*.

Alternatively, D is an all carbon saturated or unsaturated alkylene chain. In this case, D is preferably saturated and is 7 atom in length. More preferably, D is substituted with R⁴, wherein R⁴ is H, oxo, thio, hydroxy, thioalkyl, alkoxy or alkyl.

- 20 More preferably, R⁴ is H or C₁₋₆ alkyl. Most preferably, R⁴ is H or methyl. Most preferably, R⁴ is H or 10-(S)-Me.

- Alternatively, D is an all carbon alkylene chain containing preferably one double bond and is 7 atom in length. More preferably, this double bond is at position 13,14
- 25 of the chain. Most preferably, this double bond is *cis*. Preferably, this D chain is substituted with R⁴, wherein R⁴ is H, oxo, hydroxy, alkoxy or alkyl. More preferably, R⁴ is H or C₁₋₆ alkyl. Even more preferably, R⁴ is H or methyl. Most preferably, R⁴ is H or 10-(S)-Me.

A:

- 30 Preferred embodiments of the present invention include compounds of formula I as

15

described above, wherein A is a carboxylic acid.

Specific embodiments:

Preferred embodiments of the present invention include compounds of formula I as described above, wherein R² is a quinoline substituent (i.e. W is N);

- 5 R³ is a group of formula -NH-C(O)-NHR³² or -NH-C(O)-OR³²,

wherein R³² is: C₁₋₄ alkyl or C₄₋₆ cycloalkyl;

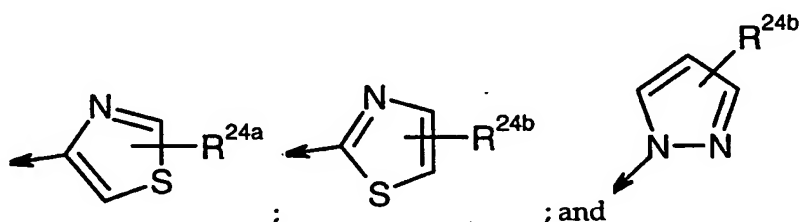
D is a 6 to 8 atom saturated or unsaturated alkylene chain linked to R¹ in configuration *syn* to A, optionally containing one or two heteroatoms independently selected from: O, S or N-R⁴¹, wherein R⁴¹ is C₂₋₇ acyl;

- 10 R⁴ is H, or from one to three substituents independently selected from hydroxy or C₁₋₆ alkyl; and

A is a carboxylic acid, or a pharmaceutically acceptable salt or ester thereof.

More preferably are compounds of formula I wherein R¹ is as defined above; R²¹ is H or methoxy;

- 15 R²² is C₁₋₆ alkoxy, or Het selected from the group consisting of:



wherein R^{24a} is H, C₁₋₆ alkyl, NH-R²⁵, NH-C(O)-R²⁵, NH-C(O)-NH-R²⁵,

wherein R²⁵ is: H, C₁₋₆ alkyl or C₃₋₆ cycloalkyl;

or R^{24a} is NH-C(O)-OR²⁶, wherein R²⁶ is C₁₋₆ alkyl or C₃₋₆ cycloalkyl;

- 20 and R^{24b} is H or C₁₋₆ alkyl;

R³ is a urea of the formula NH-C(O)-NHR³² or a carbamate of formula NH-C(O)-OR³², wherein R³² is C₁₋₆ alkyl or C₃₋₆ cycloalkyl;

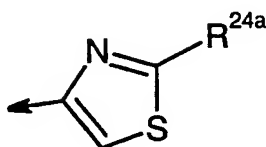
D is a C7-atom saturated or unsaturated alkylene chain optionally containing one double bond at position 11,12 or 13,14;

- 25 said D chain optionally containing one heteroatom independently selected from: O,

S, NH, N(Me), or N(Ac); and

R⁴ is H or C₁₋₆ alkyl.

Most preferably, are compounds of formula I wherein R²¹ is methoxy, and R²² is ethoxy or:



5

wherein R^{24a} is NH-(C₁₋₄ alkyl), NH-C(O)-(C₁₋₄ alkyl); or NH-C(O)-O-(C₁₋₄ alkyl);,

and

D is saturated or contains one *cis* double bond at position 13,14.

Finally, included within the scope of this invention are all compounds of formula I

10 as presented in Tables 1 to 9.

The pharmaceutical compositions of this invention may be administered orally, parenterally or via an implanted reservoir. Oral administration or administration by injection are preferred. The pharmaceutical compositions of this invention may contain any conventional non-toxic pharmaceutically-acceptable carriers, adjuvants or vehicles. In some cases, the pH of the formulation may be adjusted with pharmaceutically acceptable acids, bases or buffers to enhance the stability of the formulated compound or its delivery form. The term parenteral as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intra-articular, intrasynovial, intrasternal, intrathecal, and intralesional injection or infusion techniques.

The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example, as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to techniques known in the art using suitable dispersing or wetting agents (such as, for example, Tween 80) and suspending agents.

The pharmaceutical compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, and aqueous suspensions and solutions. In the case of tablets for oral use, carriers

which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are administered orally, the active ingredient is combined with
5 emulsifying and suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

Other suitable vehicles or carriers for the above noted formulations and compositions can be found in standard pharmaceutical texts, e.g. in "Remington's Pharmaceutical Sciences", 19th ed., Mack Publishing Company, Easton, Penn., 1995.

10 Dosage levels of between about 0.01 and about 100 mg/kg body weight per day, preferably between about 0.5 and about 75 mg/kg body weight per day of the protease inhibitor compounds described herein are useful in a monotherapy for the prevention and treatment of HCV mediated disease. Typically, the pharmaceutical compositions of this invention will be administered from about 1 to about 5 times
15 per day or alternatively, as a continuous infusion. Such administration can be used as a chronic or acute therapy. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. A typical preparation will contain from about 5% to about 95% active compound (*w/w*).
20 Preferably, such preparations contain from about 20% to about 80% active compound.

As the skilled artisan will appreciate, lower or higher doses than those recited above may be required. Specific dosage and treatment regimens for any particular patient will depend upon a variety of factors, including the activity of the specific
25 compound employed, the age, body weight, general health status, sex, diet, time of administration, rate of excretion, drug combination, the severity and course of the infection, the patient's disposition to the infection and the judgment of the treating physician. Generally, treatment is initiated with small dosages substantially less than the optimum dose of the peptide. Thereafter, the dosage is increased by small
30 increments until the optimum effect under the circumstances is reached. In general, the compound is most desirably administered at a concentration level that will generally afford antivirally effective results without causing any harmful or

deleterious side effects.

When the compositions of this invention comprise a combination of a compound of formula I and one or more additional therapeutic or prophylactic agent, both the compound and the additional agent should be present at dosage levels of between
5 about 10 to 100%, and more preferably between about 10 and 80% of the dosage normally administered in a monotherapy regimen.

When these compounds or their pharmaceutically acceptable salts are formulated together with a pharmaceutically acceptable carrier, the resulting composition may be administered *in vivo* to mammals, such as man, to inhibit HCV NS3 protease or to
10 treat or prevent HCV virus infection. Such treatment may also be achieved using the compounds of this invention in combination with agents which include, but are not limited to: immunomodulatory agents, such as α -, β -, or γ -interferons; other antiviral agents such as ribavirin, amantadine; other inhibitors of HCV NS3 protease; inhibitors of other targets in the HCV life cycle such as helicase,
15 polymerase, metalloprotease, or internal ribosome entry site (IRES); or combinations thereof. The additional agents may be combined with the compounds of this invention to create a single dosage form. Alternatively these additional agents may be separately administered to a mammal as part of a multiple dosage form.

Accordingly, another embodiment of this invention provides methods of inhibiting
20 HVC NS3 protease activity in mammals by administering a compound of the formula I, wherein the substituents are as defined above.

In a preferred embodiment, these methods are useful in decreasing HCV NS3 protease activity in a mammal. If the pharmaceutical composition comprises only a compound of this invention as the active component, such methods may
25 additionally comprise the step of administering to said mammal an agent selected from an immunomodulatory agent, an antiviral agent, a HCV protease inhibitor, or an inhibitor of other targets in the HCV life cycle such as helicase, polymerase, or metalloprotease. Such additional agent may be administered to the mammal prior to, concurrently with, or following the administration of the compositions of this
30 invention.

In an alternate preferred embodiment, these methods are useful for inhibiting viral replication in a mammal. Such methods are useful in treating or preventing HCV

disease. If the pharmaceutical composition comprises only a compound of this invention as the active component, such methods may additionally comprise the step of administering to said mammal an agent selected from an immunomodulatory agent, an antiviral agent, a HCV protease inhibitor, or an inhibitor of other targets in the HCV life cycle. Such additional agent may be administered to the mammal prior to, concurrently with, or following the administration of the composition according to this invention.

The compounds set forth herein may also be used as laboratory reagents. The Applicant provides for the first time compounds with a low molecular weight, that are highly active and specific against the HCV NS3 protease. Some of the present compounds may be instrumental in providing research tools for designing of viral replication assays, validation of animal assay systems and structural biology studies to further enhance knowledge of the HCV disease mechanisms.

The compounds of this invention may also be used to treat or prevent viral contamination of materials and therefore reduce the risk of viral infection of laboratory or medical personnel or patients who come in contact with such materials (e.g. blood, tissue, surgical instruments and garments, laboratory instruments and garments, and blood collection or transfusion apparatuses and materials).

20 Methodology

Several ways of carrying the synthesis of acyclic intermediates of compounds of formula I are disclosed in WO 00/09543 and WO 00/09558.

The compounds of the present invention are synthesized according to the general process illustrated in Schemes I, II and III (wherein PG is an appropriate protecting groups. [In all schemes presented below, D' has the same definition as D but is 2 to 5 atom shorter].

When the invention covers compounds of formula I wherein A is a N-substituted amide, vinyl-ACCA or homo-allyl ACCA (R^1) is coupled to an appropriate amine prior to the coupling to P2. Such coupling will be readily recognized by persons skilled in the art. As will be recognized by persons skilled in the art, such amide (A) is not protected but bears any relevant substituent R^5 as defined above.

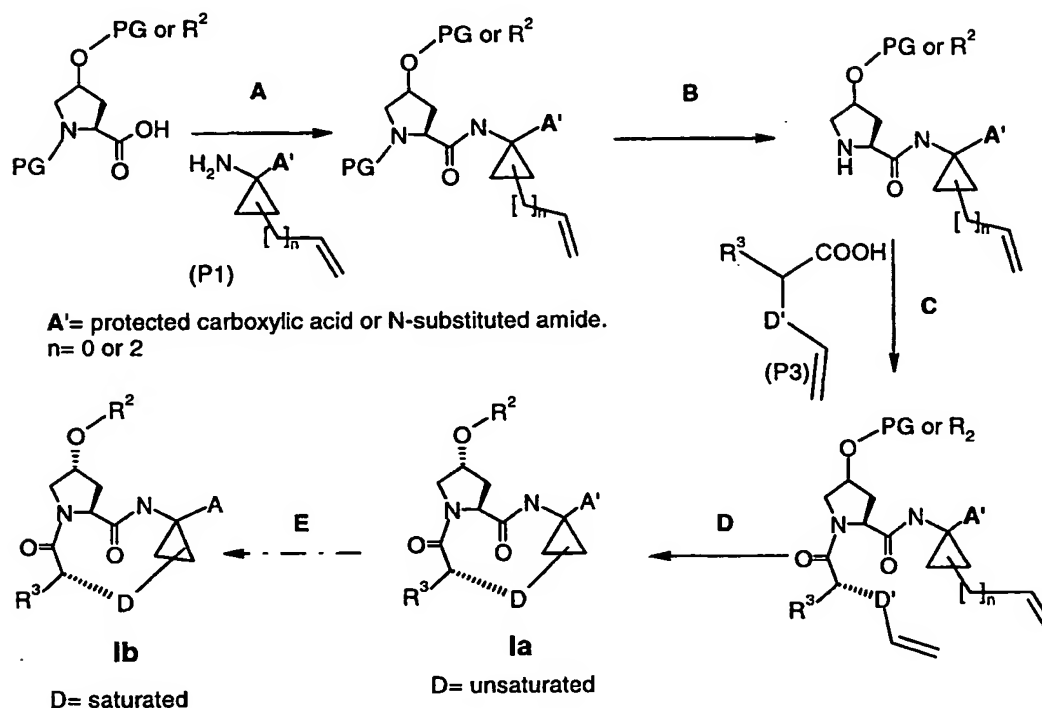
The ring-closing reaction (macrocyclization) is carried out by either olefin metathesis

(Scheme I) or when the linker contains a nitrogen atom, by reductive amination (Scheme II), or by peptide bond formation Scheme III.

Details of these processes are presented below:

5 A. Macrocyclisation via olefin metathesis

Scheme I

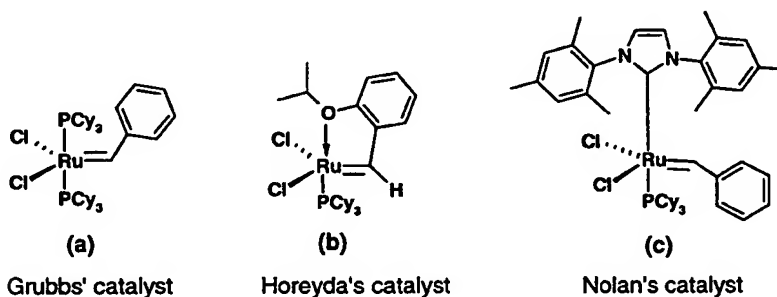


10 Scheme I:

There are several ways in which the coupling sequence can be carried out which can be easily recognized by persons skilled in the art. Starting with 4-(S)-hydroxyproline, the substituent at the 4-hydroxy can be incorporated *via* a Mitsunobu reaction (as described in Mitsunobu *Synthesis* 1981, January, 1-28; Rano *et al. Tet. Lett.* 1994, 36, 3779-3792; Krchnak *et al. Tet. Lett.* 1995, 36, 6193-6196) before or after the macrocyclization. Alternatively the assembly can be done with the required 4-(R)-hydroxy-substituted proline as disclosed in the general processes of WO 00/09543 & WO 00/09558 (see below for specific examples of these fragments).

Steps A, B, C: Briefly, the P1, P2, and P3 moieties can be linked by well known peptide coupling techniques and generally disclosed in WO 00/09543 & WO 00/09558.

- Step D: The formation of the macrocycle can be carried out via an olefin metathesis using a Ru-based catalyst such as the one reported by Miller, S.J.; Blackwell, H.E.; Grubbs, R.H. *J. Am. Chem. Soc.* 1996, 118, 9606-9614 (a); Kingsbury, J.S.; Harrity, J.P.A.; Bonitatebus, P.J.; Hoveyda, A.H. *J. Am. Chem. Soc.* 1999, 121, 791-799 (b) and Huang, J.; Stevens, E.D.; Nolan, S.P.; Petersen, J.L.; *J. Am. Chem. Soc.* 1999, 121, 2674-2678 (c). It will also be recognized that catalysts containing other transition metals such as Mo can be used for this reaction.

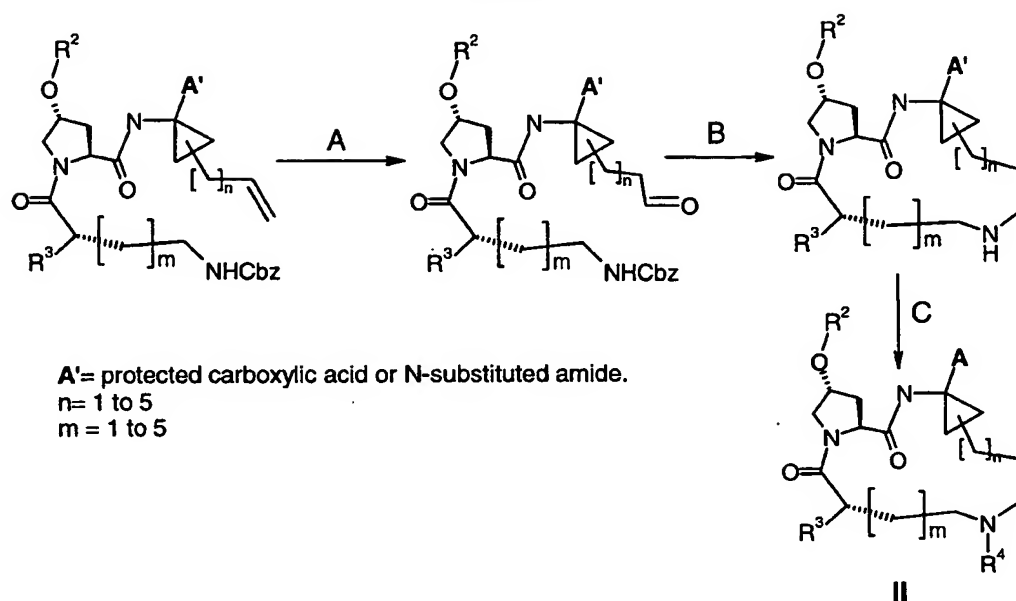


Step E: Optionally, the double bond is reduced by standard hydrogenation methods well known in the art. When A' is a protected carboxylic acid, it is also deprotected appropriately.

- 15 B. **Macrocyclization via reductive amination (for linkers containing N)**
- When the linker contains a nitrogen atom, macrocyclization is achieved by reductive amination as shown in Scheme II to obtain inhibitors of general structure II.

22

Scheme II



Step A: Hydroboration of the double bond following Brown's procedure (H.C. Brown and B.C. Subba Rao, *J. Am. Chem. Soc.* 1959, 81, 6434-6437) followed by oxidation of the resulting alcohol (for example *via* Dess-Martin periodinate, *J. Am. Chem. Soc.* 1991, 113, 7277-7287) affords the corresponding aldehyde.

Step B: Hydrogenation in the presence of acid leads to the removal of the amino protecting group followed by macrocyclization *via* reductive amination.

The P3 unit used in this synthesis is easily obtained from a variety of amino acids, such as lysine, ornithine, glutamine (after a Hofmann reaction: *Ber.* 1881, 14, 2725) and others; these synthetic modifications are methods well known in the art.

Step C: Optionally, the secondary amine in the linker D (formed after step D) is alkylated with alkyl halides or acetylated with alkyl or aryl acid chlorides using methodologies well known in the art to obtain inhibitors of general structure II.

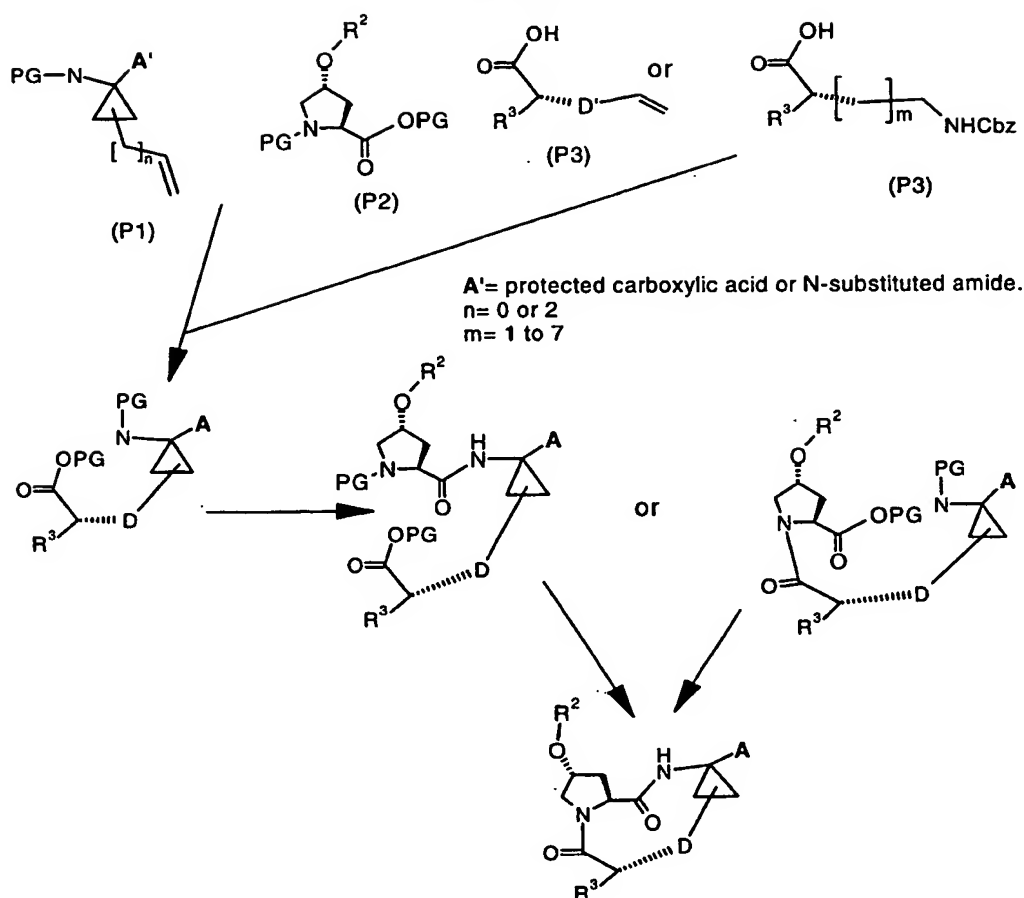
When A' is a protected carboxylic acid, it is also deprotected appropriately.

C. Macrocyclization via lactam formation

Alternatively, it is understood that these macrocyclic compounds with general structure I and II can be synthesized in other ways. For example P1 and P3 can be first connected to the linker D, then coupled to P2 and the macrocyclization reaction can be a lactam formation in two possible ways as will be recognized by persons skilled in the art and as shown in Scheme III.

23

Scheme III



Synthesis of P1

The synthesis of inhibitors with general structure I and II requires the same P1

5 fragments:

- a) vinyl ACCA, the synthesis and resolution of which is described in WO 00/09543 & WO 00/09558 or
- b) homoallyl ACCA (Example 1, compound 1f).

Synthesis of P2

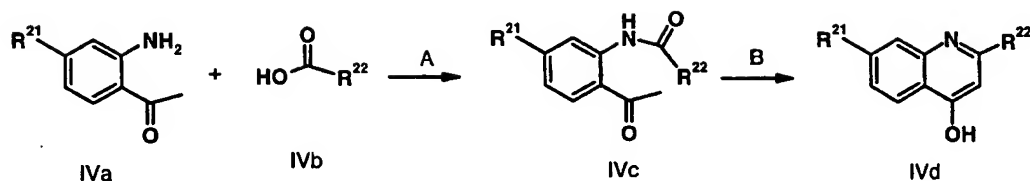
10 Some of the P2 fragments used for the synthesis of compounds of formula I are described in WO 00/09543 & WO 00/09558.

Other P2 fragments are synthesized as follows:

a. Synthesis of 2-"Het"-4-hydroxy-7-methoxyquinoline derivative

15 (i) Approach from the corresponding "Het" carboxylic acid IVb

Scheme IV



The synthesis is carried out according to a modified procedure in Li *et al.* *J. Med.*

- 5 *Chem.* **1994**, *34*, 3400-3407. Intermediate IVa where R²¹ = OMe (Example 7, compound 7b) is prepared as described by Brown *et al.* *J. Med. Chem.* **1989**, *32*, 807-826.

Step A: Intermediate IVa is coupled with heterocyclic carboxylic acids IVb under basic conditions with POCl₃ to activate the carboxylate group. A variety of

- 10 carboxylic acids with general structure IVb are used for the preparation of inhibitors; these are either commercially available, synthesized as shown in scheme V, VI and VII, or synthesized individually using methods described in the specific examples.

Step B: Ring-closure, followed by dehydration is achieved under basic conditions to obtain quinolines of general structure IVd.

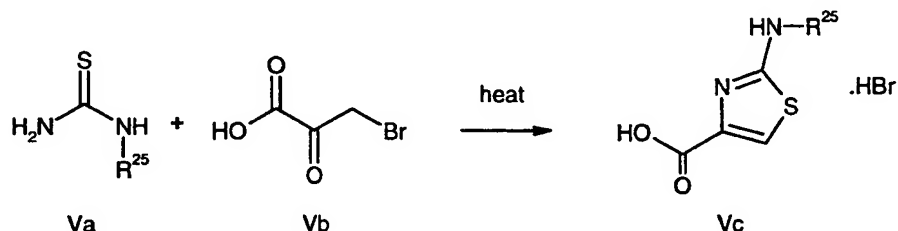
15

(i.a). **Synthesis of "Het"-carboxylic acids of general formula IVb**
Synthesis of 2-(substituted)-amino-4-carboxy-aminothiazole derivatives (Vc)

The modified procedure described by Berdikhina *et al.* *Chem. Heterocycl. Compd.*

- 20 (Engl. Transl.) **1991**, *4*, 427-433 is used.

Scheme V



- A variety of 2-alkylaminothiazolyl-4-carboxylic acids, compounds of general structure Vc, are made using the general synthetic methodology outlined in Scheme
- 25

The starting carboxylic acids (Xa) is commercially available or is prepared by know literature procedures familiar to those skilled in the art.

Step A: The carboxylic acid Xa is activated with pivaloyl chloride and then reacted with the anion of Evans' chiral auxiliary 4(S)-4-(phenylmethyl)-2-oxazolidinone
5 following well known chemistry (Review: D.J. Ager *et al. Aldrichimica Acta* 1997, 30, 3-11, and references therein) to obtain compounds of general structure Xb.

Step B: Stereoselective α -azidation with trizylazide, of a chiral imide enolate such as those which would form from compounds with general structure Xb in the presence of a base like KHMDS, is also well known in the art (Review: D.J. Ager *et al.*
10 *Aldrichimica Acta* 1997, 30, 3-11, and references therein).

Step C: Reduction of the α -azide, catalyzed by SnCl_2 , is followed by protection of the amine formed as its t-butyl carbamate gives intermediates of general structure Xc.

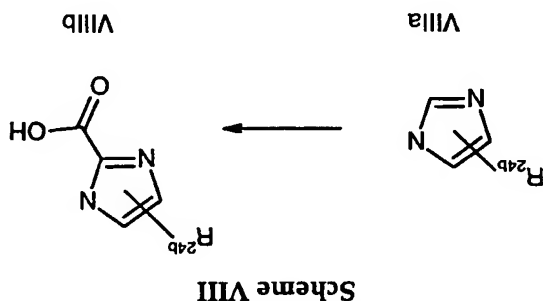
These reactions are also well known in the art.

Step D: Finally, the chiral auxiliary is hydrolyzed under basic conditions, such as a
15 mixture of H_2O_2 with LiOH, to produce the amino acid-type linkers of general structure Xe.

Alternatively, P3 moieties having the same general structure Xe are synthesized following de procedure described by M.J. Burk *et al. J. Am. Chem. Soc* 1998, 120, 657-
20 663 illustrated in Scheme XI. These compounds varied in the number of methylene units ($-\text{CH}_2-$) along the linker ($m=1$ to 5) and the substitution of alkyl groups at R_4 , but did not contain a heteroatom.

Synthesis of 2-carboxy-(substituted)-imidazole derivative
(VIIIb)

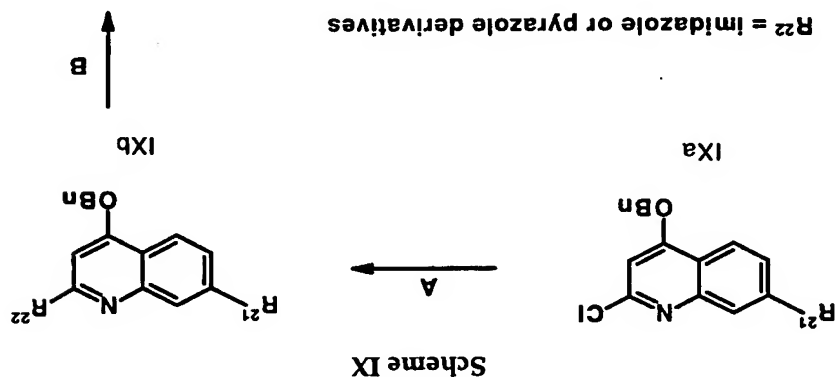
A variety of alkylimidazole-2-carboxylic acids, compounds of general structure VIIIb, are made using the general synthetic methodology outlined in Scheme VIII.



The procedure described by Baird *et al.*, *J. Amer. Chem. Soc.* 1996, 118, 6141-6146, was used: an alkyl imidazole is deprotonated with a strong base (e.g. *n*BuLi) and then reacted with CO₂ to form the carboxylic acid VIIIb. This type of condensation

reaction is well known in the art.

b. **Synthesis of 4-hydroxy-7-methoxy-2-(imidazolyl or pyrazolyl)quinolines**
 4-Hydroxy-7-R²¹ quinolines having an imidazolyl or pyrazolyl moiety at C2 are generally prepared using the methodology outlined in Scheme IX.



The synthesis of the key intermediate, (wherein R²¹ = OMe) 4-benzyloxy-2-chloro-7-methoxyquinoline IXa is described in detail in Example 6 (compound 6e).

different alkyl substituents (R^{25} = alkyl group) and 3-

type of condensation reaction is well known in the art.

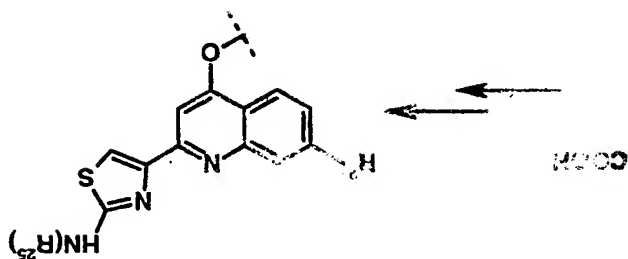
ment containing the 2-amino-substituted-thiazole

from the corresponding 2-carboxyl derivative as shown

the procedure of Urangst, P.C.; Connor, D.T. J. *Heterocyc.*

10.

Scheme VI

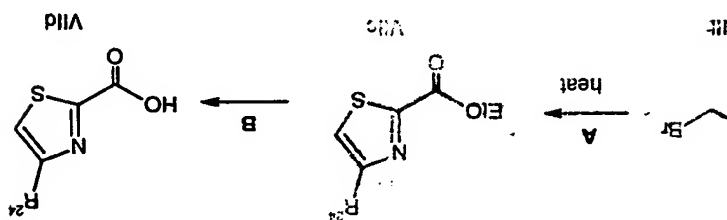


11. *Journal of Heterocyclic Chemistry*, 24(4): 555-558, 1988.

12. *Journal of Heterocyclic Chemistry*, 24(4): 555-558, 1988.

general structure

in Scheme VII.



by Urangst et al. J. *Med. Chem.* 1998, 41, 3515-3529 is used

described as follows: Ethyl thiooxamate (VIIa) is reacted with

of general structure VIIb (R^{24} = alkyl group) to form

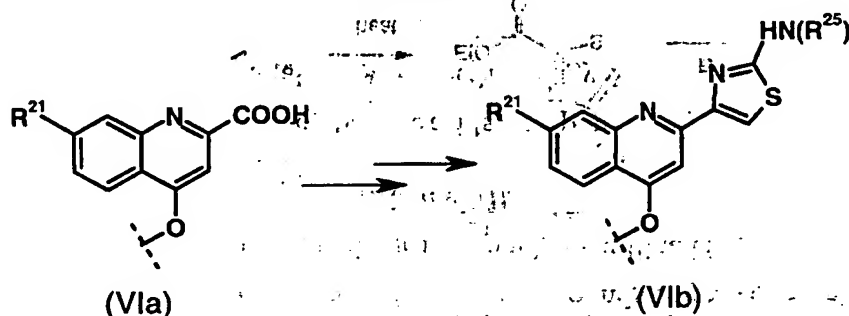
of general structure VIIc. This type of condensation

the art.

25

V using thioureas (Va) with different alkyl substituents (R^{25} = alkyl group) and 3-bromopyruvic acid. This type of condensation reaction is well known in the art. Alternatively, the P2 fragment containing the 2-amino-substituted-thiazole derivatives are synthesized from the corresponding 2-carboxyl derivative as shown in scheme VI according to the procedure of: Unangst, P.C.; Connor, D.T. *J. Heterocyc. Chem.* 29, 5, 1992, 1097-1100.

Scheme VI

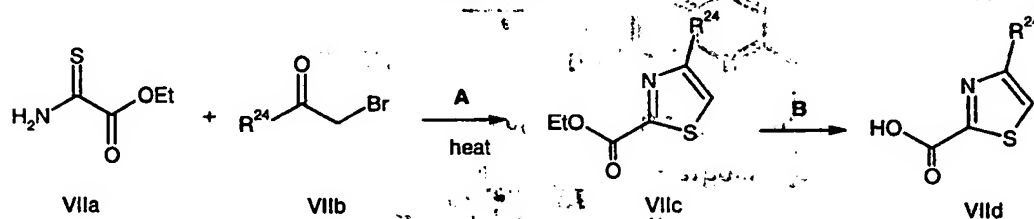


Examples of this process are disclosed in WO 00/09543 & WO 00/09558.

Synthesis of 2-carboxy-4-substituted aminothiazole derivatives VIId

A variety of 4-alkylthiazolyl-2-carboxylic acids, compounds of general structure VIId, is made using the general synthetic methodology outlined in Scheme VII.

Scheme VII



The procedure described by Janusz *et al.* *J. Med. Chem.* 1998, 41, 3515-3529 is used with modifications as described as follows: Ethyl thiooxamate (VIIa) is reacted with different β -bromoketones of general structure VIIb (R^{24} = alkyl group) to form thiazolyl carboxylic acids of general structure VIId. This type of condensation reaction is well known in the art.

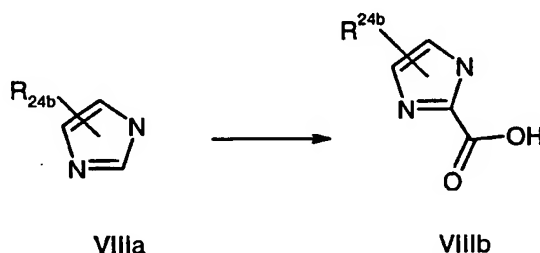
52

Synthesis of 2-carboxy-(substituted)-imidazole derivative
(VIIIb)

A variety of alkylimidazolyl-2-carboxylic acids, compounds of general structure VIIIb, are made using the general synthetic methodology outlined in Scheme VIII.

5

Scheme VIII

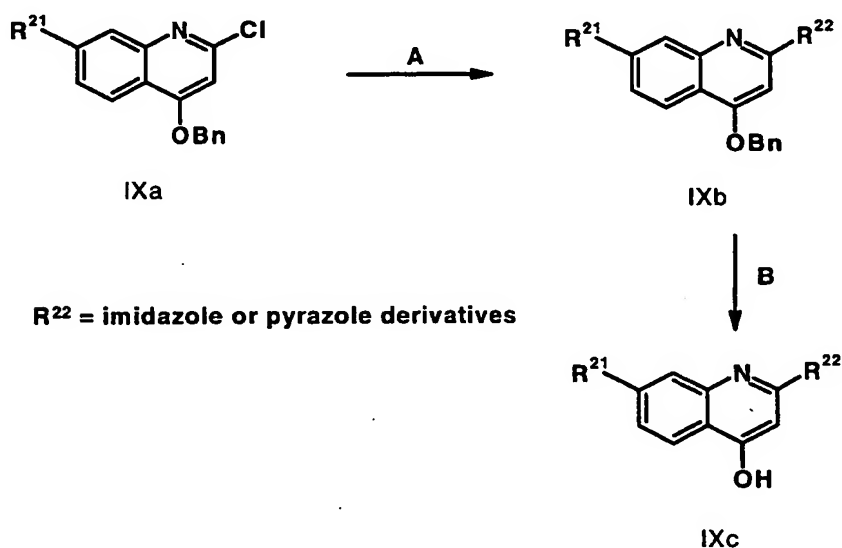


The procedure described by Baird *et al.* *J. Amer. Chem. Soc.* 1996, 118, 6141-6146. was used: an alkyl imidazole is deprotonated with a strong base (e.g. *n*BuLi) and then reacted with CO₂ to form the carboxylic acid VIIIb. This type of condensation reaction is well known in the art.

10

b. **Synthesis of 4-hydroxy-7-methoxy-2- (imidazolyl or pyrazolyl)quinolines**
4-Hydroxy-7-R²¹ quinolines having an imidazolyl or pyrazolyl moiety at C2 are generally prepared using the methodology outlined in Scheme IX.

Scheme IX



15

The synthesis of the key intermediate, (wherein R²¹ = OMe) 4-benzyloxy-2-chloro-7-methoxyquinoline IXa is described in detail in Example 6 (compound 6e).

Step A: At high temperatures, a variety of imidazoles, alkyl substituted imidazoles, pyrazoles or alkyl substituted pyrazoles can be used to displace the 2-chloro moiety of compound IXa giving compounds of general structure IXb.

Step B: Upon removal of the benzyl protecting group from the 4-hydroxy moiety of the quinoline by standard hydrogenation methods, quinoline derivatives of general structure IXc are obtained.

Synthesis of P3

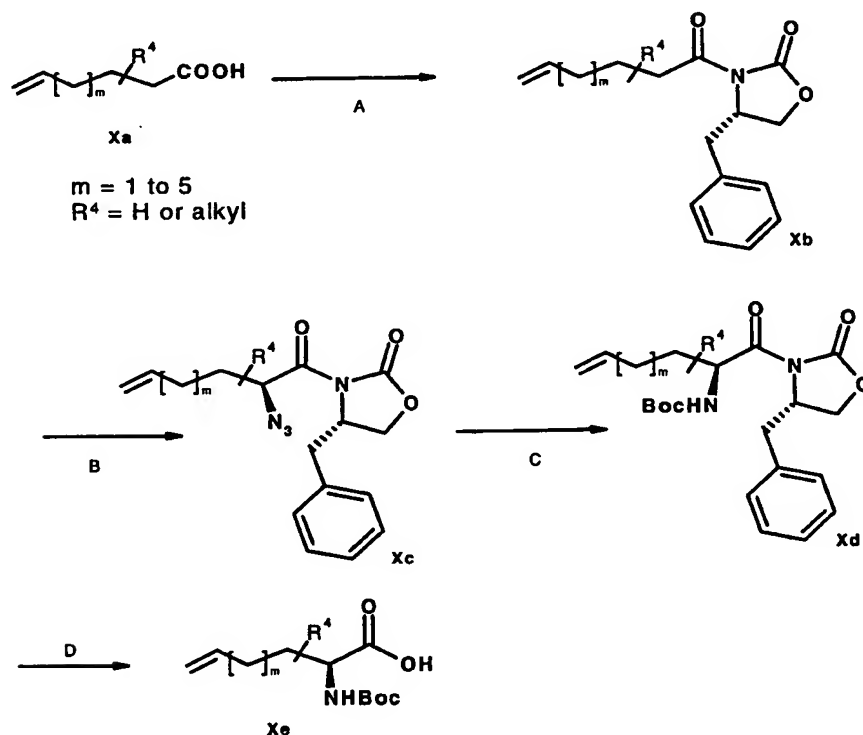
A variety of P3 fragments are synthesized containing the appropriate D linker extension for macrocyclization by olefin metathesis. In general P3 units containing a terminal olefin for metathesis are synthesized following the general schemes shown below (Schemes X, XI & XII).

Synthesis of Linkers in Class A

This general synthesis is used to make linkers that are all carbon based (no heteroatom) (Scheme X).

15

Scheme X



The synthesis is performed according to the procedure of Evans *et al.* *J. Am. Chem. Soc.* 1990, 112, 4011-4030.

The starting carboxylic acids (Xa) is commercially available or is prepared by know literature procedures familiar to those skilled in the art.

Step A: The carboxylic acid Xa is activated with pivaloyl chloride and then reacted with the anion of Evans' chiral auxiliary 4(S)-4-(phenylmethyl)-2-oxazolidinone
5 following well known chemistry (Review: D.J. Ager *et al. Aldrichimica Acta* 1997, 30, 3-11, and references therein) to obtain compounds of general structure Xb.

Step B: Stereoselective α -azidation with trizylazide, of a chiral imide enolate such as those which would form from compounds with general structure Xb in the presence of a base like KHMDS, is also well known in the art (Review: D.J. Ager *et al.*
10 *Aldrichimica Acta* 1997, 30, 3-11, and references therein).

Step C: Reduction of the α -azide, catalyzed by SnCl_2 , is followed by protection of the amine formed as its t-butyl carbamate gives intermediates of general structure Xc.

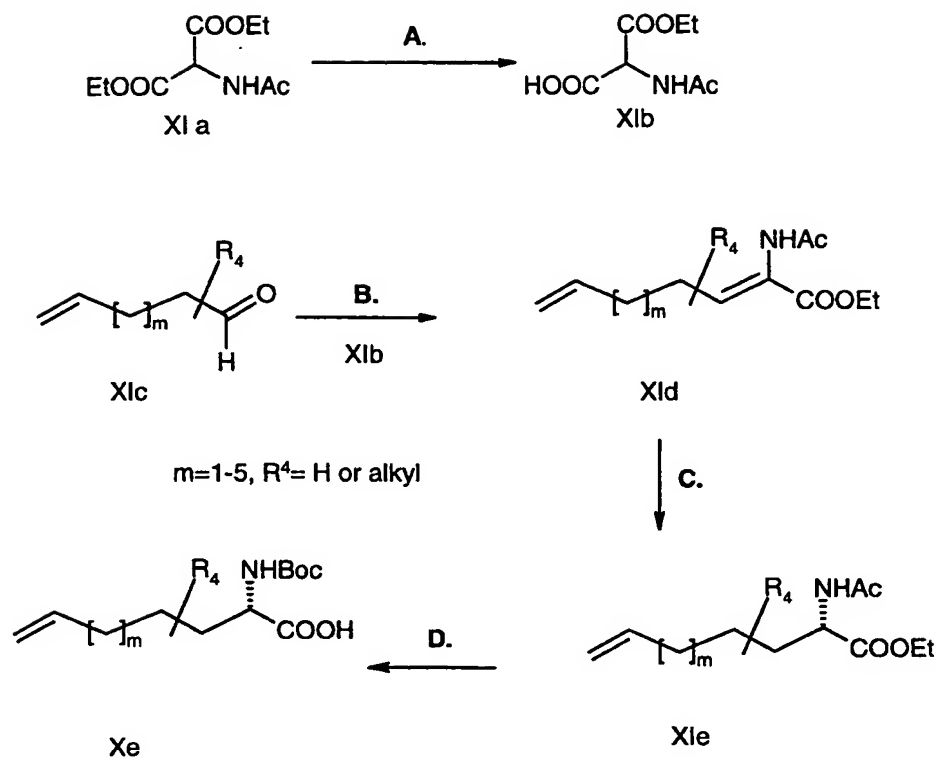
These reactions are also well known in the art.

Step D: Finally, the chiral auxiliary is hydrolyzed under basic conditions, such as a
15 mixture of H_2O_2 with LiOH, to produce the amino acid-type linkers of general structure Xe.

Alternatively, P3 moieties having the same general structure Xe are synthesized following de procedure described by M.J. Burk *et al. J. Am. Chem. Soc* 1998, 120, 657-
20 663 illustrated in Scheme XI. These compounds varied in the number of methylene units ($-\text{CH}_2-$) along the linker ($m=1$ to 5) and the substitution of alkyl groups at R_4 , but did not contain a heteroatom.

29

Scheme XI



Step A: The monoacid compound XIb is prepared from commercially available diethyl 2-acetamidomalonate by standard ester hydrolysis under basic conditions.

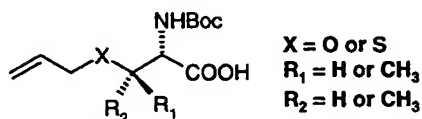
- 5 **Step B:** Knoevenagel-type condensation between an aldehyde of general structure XIc and compound XIb in the presence of a base, such as pyridine, and acetic anhydride leads to the formation of enamide intermediate XIId having the Z stereochemistry around the newly formed double bond as shown.

- 10 **Step C:** Regioselective and enantioselective catalytic hydrogenation of the enamide intermediate XIId to the amino acid intermediate XIe is achieved using Burk's method.

- Step D:** The nitrogen of the acetamido derivative XIe is then di-protected with the addition of a t-butyl carbamate substituent before the acetate group, as well as the ethyl ester, are hydrolyzed under standard basic condition to obtain P3 moieties of
- 15 general structure XIIf.

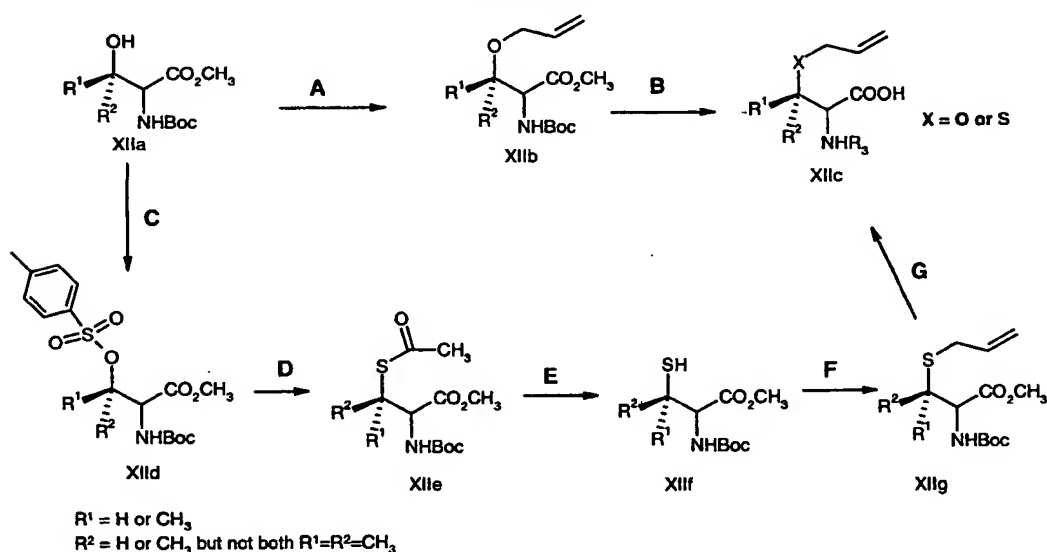
Synthesis of Linkers in Class B

General Structure of Linkers in Class B



This general synthesis is used to make linkers containing oxygen or sulfur.

Scheme XII



5

Step A: Suitably protected amino acids, such as Boc-(L)-serine methyl ester, Boc-(L)-threonine methyl ester or Boc-(L)-allothreonine methyl ester, are alkylated with allyl iodide in the presence of Ag_2O to give the methyl ester XIIb.

10 **Step B:** Hydrolysis of the methyl ester under standard basic conditions yields the ether-type linkers of general structure XIIc ($X=O$).

Step C: The sulfur analog is prepared from the same starting amino acid XIIa (appropriately protected as before) and its hydroxyl group is converted to a good leaving group (such as the tosylate intermediate XIIId) using standard methodology well known in the art.

15 **Step D:** The tosyl moiety is subsequently displaced with the anion of thioacetate leading to the formation of the thioester intermediate XIIe by inversion of the chiral center at the β -carbon.

31

Step E: Hydrolysis of the thioester moiety under mild basic conditions yields the free thiol XII_f.

Step F: Alkylation of the thiol moiety is easily achieved under basic conditions with allyl iodide.

- 5 Step G: Finally, the sulfide analog XII_c (X=S) are obtained after hydrolysis of the methyl ester using standard procedures.

Synthesis of R³ fragment:

Examples of synthesis of fragments wherein R³ is NH-R³¹ are disclosed in WO 00/09543.

10

EXAMPLES

The present invention is illustrated in further detail by the following non-limiting examples. Other specific ways of synthesis or resolution can be found in WO 00/09543 & WO 00/09558.

- 15 Temperatures are given in degrees Celsius. Solution percentages express a weight to volume relationship, and solution ratios express a volume to volume relationship, unless stated otherwise. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 400 MHz spectrometer; the chemical shifts (δ) are reported in parts per million and are referenced to the internal deuterated solvent unless otherwise
- 20 indicated. The NMR spectra of all final compounds (inhibitors) was recorded in DMSO-d₆ of their TFA salt unless otherwise indicated. Flash column chromatography was carried out on silica gel (SiO₂) according to Still's flash chromatography technique (W.C. Still et al., *J. Org. Chem.*, 1978, 43, 2923). Abbreviations used in the examples include Bn: benzyl; Boc: *tert*-butyloxycarbonyl
- 25 [Me₃COC(O)]; BSA: bovine serum albumin; Cbz: benzyloxycarbonyl; CHAPS: 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate; DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene; CH₂Cl₂= DCM: methylene chloride; DEAD: diethylazodicarboxylate; DIAD: diisopropylazodicarboxylate; DIPEA: diisopropylethylamine; DMAP: dimethylaminopyridine; DCC: 1,3-dicyclohexylcarbodiimide;
- 30 DME: 1,2-dimethoxyethane; DMF: dimethylformamide; DMSO: dimethylsulfoxide; DTT: dithiothreitol or threo-1,4-dimercapto-2,3-

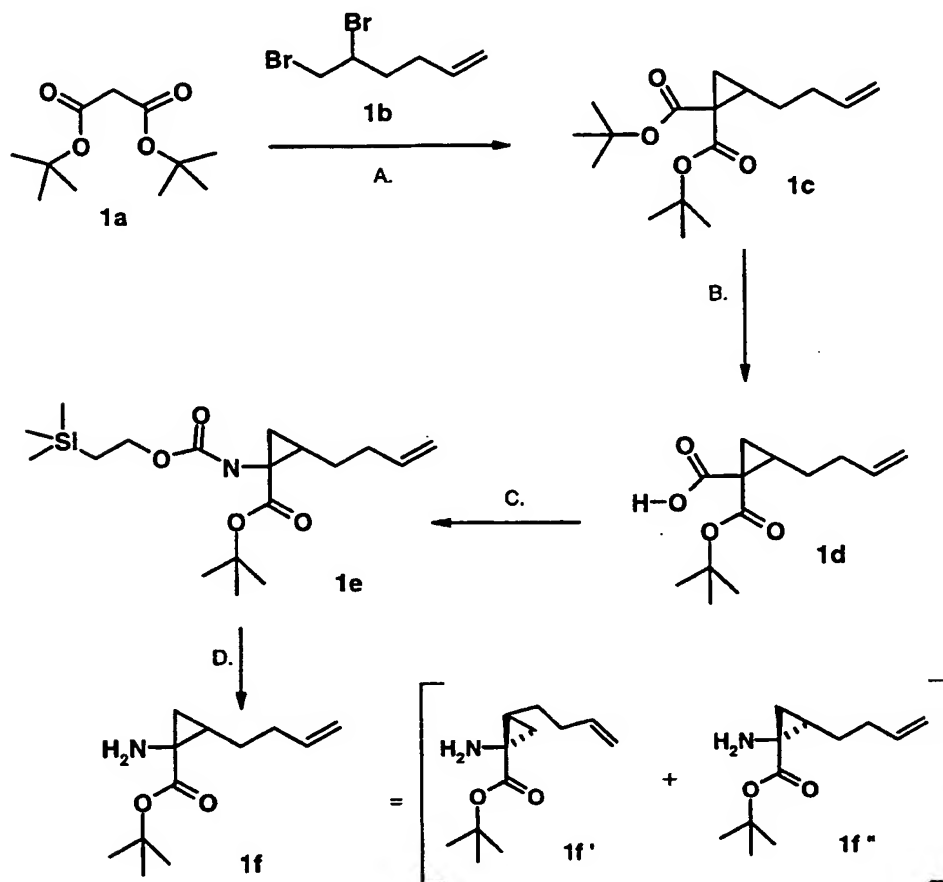
32

butanediol; DPPA: diphenylphosphoryl azide; EDTA: ethylenediaminetetraacetic acid; Et: ethyl; EtOH: ethanol; EtOAc: ethyl acetate; Et₂O: diethyl ether; ESMS: electrospray mass spectrometry; HATU: O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HPLC: high performance liquid chromatography; MS: mass spectrometry; MALDI-TOF: Matrix Assisted Laser Disorption Ionization-Time of Flight, FAB: Fast Atom Bombardment; LAH: lithium aluminum hydride; Me: methyl; MeOH: methanol; MES: (2-[N-morpholino]ethanesulfonic acid); NaHMDS: sodium bis(trimethylsilyl)amide; NMM: N-methylmorpholine; NMMO: N-methylmorpholine oxide; NMP: N-methylpyrrolidine; Pr: propyl; Succ: 3-carboxypropanoyl; PNA: 4-nitrophenylamino or p-nitroanilide; TBAF: tetra-n-butylammonium fluoride; TBME: *tert*-butyl-methyl ether; *t*BuOK: potassium *tert*-butoxide; TBTU: 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TCEP: tris(2-carboxyethyl) phosphine hydrochloride; TFA: trifluoroacetic acid; THF: tetrahydrofuran; TIS: triisopropylsilane; TLC: thin layer chromatography; TMSE: trimethylsilylethyl; Tris/HCl: tris(hydroxymethyl)aminomethane hydrochloride.

P1 MOIETIES

EXAMPLE 1

Synthesis of *t*-butyl-(1*R*,2*R*)/(1*S*,2*S*)-1-amino-2-homoallylcyclopropyl carboxylate (1*f*):



5

A. To a suspension of benzyltriethylammonium chloride (5.08 g, 22.3 mmol.) in 50% aqueous NaOH (50 mL), 1,2-dibromo-5-hexene (**1b**, 8.10 g, 33.46 mmol) and di-*t*-butylmalonate (**1a**, 4.82 g, 22.30 mmol) were added in succession. The mixture was stirred vigorously at RT for 16 h, then diluted with H₂O and extracted with CH₂Cl₂

10 (3 x 50 mL). The organic layer was further washed with H₂O (2 x 50 mL), brine/H₂O (2/1, 2 x 50 mL, dried over MgSO₄ and evaporated. The crude residue was purified by flash column chromatography on silica gel, using 3 to 5% EtOAc in hexane as the eluent, to obtain compound **1c** in 38% yield (2.48 g).

¹H NMR (CDCl₃, 400 MHz): δ 1.19 (bd, *J* = 7.9 Hz, 2H), 1.25-1.33 (m, 1H), 1.46 (s, 9H),

34

1.48 (s, 9H), 1.47-1.60 (m, 1H), 1.75-1.82 (m, 1H), 2.14-2.22 (m, 2H), 4.93-5.50 (m, 2H), 4.96 (dm, $J = 10.2$ Hz, 1H), 5.18 (dm, $J = 17.2$ Hz, 1H). ES(+)MS m/z 297 (M+H)⁺.

B. To a suspension of potassium t-butoxide (5.75 g, 51.25 mmol) in anhydrous diethyl ether (150 mL) at 0°, H₂O was added (203 µL, 11.27 mmol) and the reaction mixture was stirred at 0° for 10 min. An ether solution of compound 1c (2.48 g in 10 mL diethyl ether, 10.25 mmol) was added and the mixture was stirred at RT for 5 h. The mixture was diluted with ice-cold H₂O and extracted with diethyl ether (3 x 200 mL). The aqueous layer was acidified to pH 3.5-4 with ice-cold 10% aqueous citric acid and re-extracted with EtOAc (3 x 200 mL). The EtOAc layer was washed with H₂O (2 x 100 mL), brine (100 mL), dried over MgSO₄ and evaporated to give compound 1d in 85% yield based on the amount of recovered starting material.

¹H NMR (CDCl₃, 400 MHz): δ 1.51 (s, 9H), 1.64-1.68 (m, 1H), 1.68-1.75 (m, 1H), 1.77-1.88 (m, 1H), 1.96-2.01 (m, 1H), 2.03-2.22 (m, 3H), 5.01 (dm, $J = 6.4$ Hz, 1H), 5.03 (dm, $J = 14.9$ Hz, 1H), 5.72-5.83 (m, 1H). ES(+)MS: m/z 241 (M+H)⁺.

C. To a solution of the acid 1d in anhydrous benzene (1.14 g in 25 mL benzene, 4.74 mmol), Et₃N (800 µL, 5.68 mmol) was added, followed by the addition of diphenylphosphoryl azide (1.13 mL, 5.21 mmol) and the mixture was heated to reflux for 3.5 h. Subsequently, trimethylsilylethanol (1.36 mL, 9.48 mmol) was added and stirring at reflux was continued for an additional 4 h. The mixture was then cooled to RT, evaporated to half of its original volume, diluted with diethyl ether (30 mL) and washed with 5% aqueous NaHCO₃ (2 x 30 mL), brine (50 mL), dried over MgSO₄ and evaporated. The residual oil was chromatographed on silica gel using 10% EtOAc in hexane as the eluent to obtain pure compound 1e in 88% yield (1.49 g).

¹H NMR (CDCl₃, 400 MHz) δ 0.03 (s, 9H), 0.91-0.99 (m, 2H), 1.18-1.29 (m, 2H), 1.45 (bs, 11H), 1.56-1.72 (m, 2H), 2.02-2.18 (m, 2H), 4.12 (t, $J = 8.3$ Hz, 2H), 4.93 (dm, $J = 10.2$ Hz, 1H), 4.98 (dm, $J = 17.2$ Hz, 1H), 5.07 (bs, 1H), 5.71-5.83 (m, 1H).

D. To a solution of the cyclopropyl derivative 1e (1.19 g, 3.35 mmol, in 30 mL

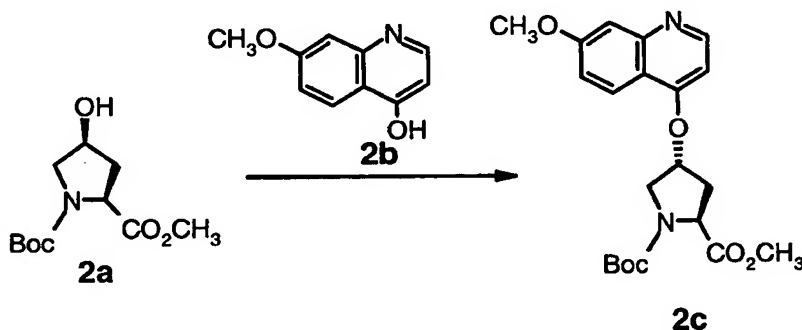
35

THF), $t\text{-Bu}_4\text{NF}$ (6.7 mL of 1M in THF, 6.7 mmol.) was added and the mixture was first stirred at RT for 16 h and subsequently heated to reflux for 15 min. The solvent was carefully evaporated under low pressure (due to the high volatility of the free amine **1f**, caution should be exercised during the evaporation of the solvent). The crude residue was re-dissolved in EtOAc (100 mL) and washed with H_2O (2x 50mL),
 5 brine (50mL), dried over MgSO_4 and again the solvent was carefully evaporated. The crude product **1f** (as a mixture of two enantiomers **1f'** and **1f''**) was used for coupling with the P2 proline derivatives without further purification. Isolation of the P1P2 fragment having the desired stereochemistry at P1 was easily achieved at this
 10 stage using flash chromatography (example 21, fragment **21b**).

P2 MOIETIES

EXAMPLE 2

Synthesis of Boc-4(R)-[(7-methoxy-4-quinolinyloxy)proline (**2c**):



15

4-Hydroxy-7-methoxyquinoline (**2b**) was prepared according to the method described by Chun, M.W.; Olmstead, K.K.; Choi, Y.S.; Lee, C.O.; Lee, C.-K.; Kim, J.H.; Lee, J. *Bioorg. Med. Chem. Lett.* 1997, 7, 789. A solution of compound **2b** (1.88 g, 10.73 mmol) and DEAD (3.4 mL, 21.46 mmol) in anhydrous THF were added to a stirring solution of protected *cis*-hydroxyproline **2a** (2.63 g, 10.73 mmol) and triphenylphosphine (5.63 g, 21.46 mmol) in anhydrous THF (160 mL) at 0° under N_2 .
 20 The reaction mixture was allowed to warm-up to RT and stir for 14 h. The THF was then evaporated and the pure product **2c** was isolated after flash column chromatography using 5% MeOH in EtOAc as the eluent, in 35% yield (1.5 g).

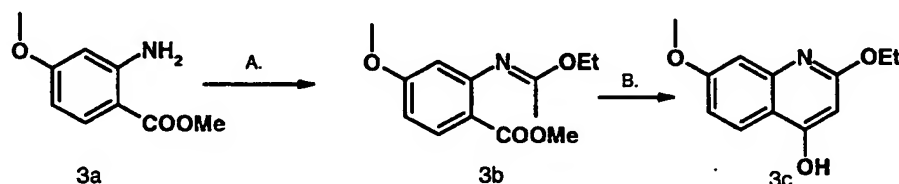
25 ^1H NMR (CDCl_3 , 400 MHz): δ 1.44 (s, 9H), 1.65 (bs, 1H), 2.34-2.43 (m, 1H), 2.63-2.76

36

(m, 1H), 3.78 (s, 3H), 3.75-3.85 & 3.89-3.99 (2m, 1H, 2 rotamers), 3.95 (s, 3H), 4.51 & 4.60 (2t, $J = 8$ Hz, 1H, 2 rotamers), 5.15 (bs, 1H), 6.53-6.59 (m, 1H), 7.12-7.18 (dd, $J = 8.9$ & 2.2 Hz, 1H), 7.36 (d, $J = 2.6$ Hz, 1H), 8.03 (bd, $J = 9.2$ Hz, 1H), 8.65 (bd, $J = 5.1$ Hz, 1H).

5 EXAMPLE 3

Synthesis of 2-ethoxy-4-hydroxy-7-methoxy quinoline (3c)



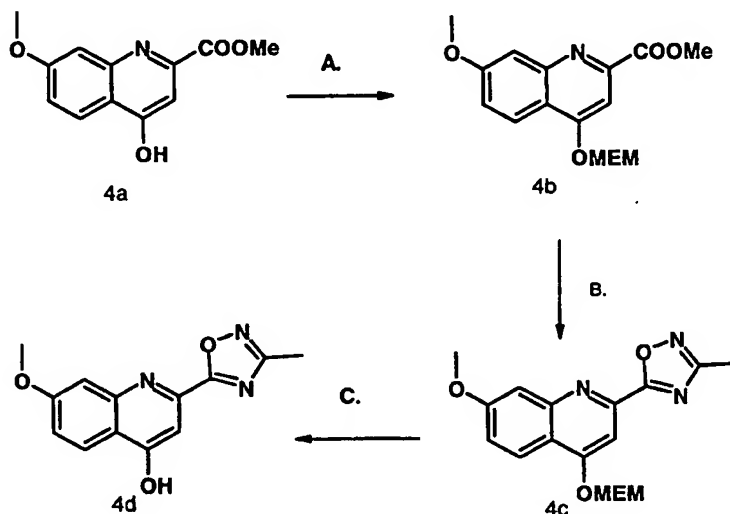
The synthesis of Methyl-*p*-methoxyantranilate 3a was done as described in Katz *et al. J. Org. Chem.*, 1953, 18, 1380-1400.

- 10 The general synthesis for the quinoline derivative 3c is a modification of the procedure of Baccar *et al. Indian Journal of Chemistry*, 1995, Sat. B, 330-332.

A. Methyl-*p*-methoxyantranilate 3a (3.069 g, 16.96 mmol) was dissolved in triethylorthoacetate (4.7 mL, 25.4 mmol), then a solution of anhydrous HCl (4 N/Dioxane, 50 μ L, 0.6 mmol) was added. The resulting mixture was heated at
15 reflux for 19 hours. The volatiles were then evaporated under vacuum to give product 3b (4.92 g, amber oil, quantitative yield) that was used as such for the next step.

B. To a solution of the substrate 3b (assumed 16.96 mmol) in THF (34 mL) at -78°C under nitrogen, was added LiHMDS (1 M/THF, 22 mL, 1.3 eq.). Shortly after
20 the addition, the cold temperature bath was removed and the mixture was left to stir at ambient temperature for 1 hour, after which time, another portion of LiHMDS (16 mL) was added. The resulting mixture was stirred until complete disappearance of starting material (1 hour) by TLC (100% EtOAc, imidate $R_f = 0.7$, product $R_f = 0.2$).
HCl (4 N/dioxane, 10 mL) was then added and the mixture was concentrated under
25 vacuum. The resulting paste was triturated from a mixture of EtOAc (10 mL) and aqueous NaH_2PO_4 (1 M, 10 mL) and sonicated. An abundant precipitate was formed, collected by filtration, washed with water and dried to afford the desired product 3c as a beige solid (3.117 g, 84% yield for 2 steps, >99% purity by HPLC).

¹H NMR (400 MHz, DMSO-d) δ (ppm): 7.88 (d, J = 8.9 Hz, 1H), 6.98 (br. s, 1H), 6.89 (br. d, J = 8.6 Hz, 1H), 5.94 (br. s, 1H), 4.30 (br. s, 2H), 3.84 (s, 3H), 1.34 (t, J = 7.0 Hz, 3H).

EXAMPLE 4**5 Synthesis of 4-hydroxy-7-methoxy-2(3-methyl-1,2,4-oxadiazol-5-yl) quinoline (4d)**

A. To a solution of 2-carbomethoxy-4-hydroxy-7-methoxyquinoline 4a (the preparation of which is described in WO 00/09543 and WO 00/09558) (1 g, 4.29 mmol) in DMF (10 mL) under nitrogen was added NaH (60% in mineral oil, 190 mg, 4.98 mmol). The resulting mixture was stirred at ambient temperature for 1 hour, MEM chloride (455 μ L, 4.98 mmol) was then added dropwise and the resulting mixture was stirred at ambient temperature for an extra 19.5 hours. The reaction mixture was diluted with EtOAc (100 mL), washed with H₂O (50 mL), brine (50 mL), dried with MgSO₄, concentrated under vacuum to afford the crude reaction isolate (1.37 g). The latter was purified by flash column chromatography to afford product 4b (1.04 g, 75% yield) as a colorless oil.

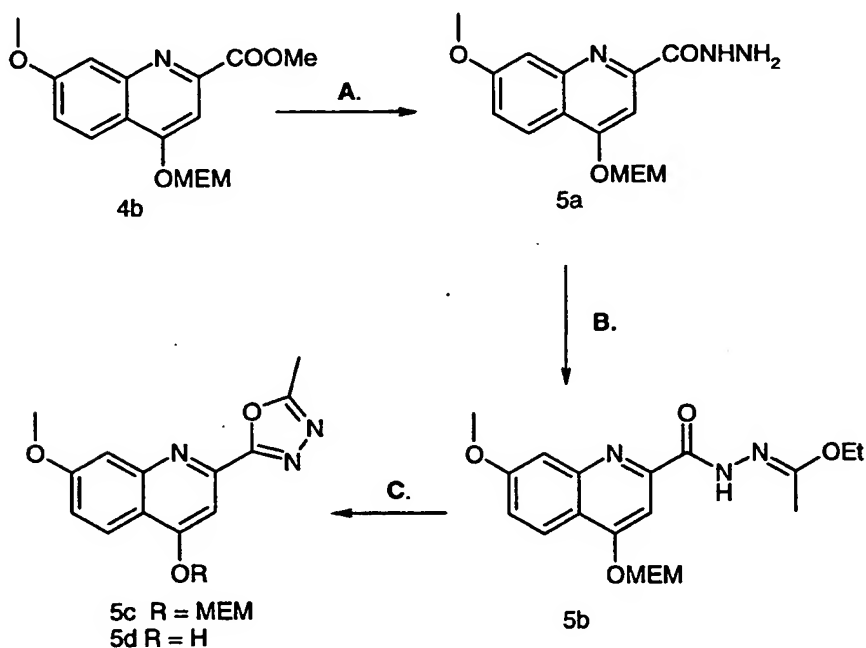
B. To a mixture of freshly activated 4Å molecular sieve (500 mg) and acetamidoxime (248 mg, 3.35 mmol) was added THF (3mL). The resulting mixture was stirred for 15 min. under nitrogen at ambient temperature, then NaH (60% in mineral oil, 124 mg, 3.24 mmol) was added by portions. The resulting suspension was stirred at ambient temperature for 1 hour, then ester 4b (500 mg, 1.56 mmol) was added in solution in THF (5 mL). The resulting mixture was heated at reflux for

1 hour then filtered over Celite, rinsing with EtOAc (3 portions of 20 mL) and concentrated under vacuum. The resulting crude mixture was purified by flash column chromatography (100% EtOAc) to afford product 4c (352 mg, 65% yield) as a white solid.

- 5 C. To the MEM ether 4c (170 mg, 0.493 mmol) in THF (4 mL) was added aqueous HCl (1 N, 1 mL). The resulting mixture was stirred at ambient temperature for 1 hour then diluted with aqueous NaH_2PO_4 (1 M, 50 mL). The solid formed was filtered, triturated with EtOAc, filtered and dried to afford the desired product (4d) (90 mg, 71% yield) as a white solid. MS (ES+) 258 (M+1), (ES-) 256 (M-1).
- 10 ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 8.03 (d, J = 9.2 Hz, 1H), 7.38 (d, J = 2.2 Hz, 1H), 7.06 (d, J = 8.6 Hz, 1H), 6.85 (br. s, 1H), 3.88 (s, 3H), 2.64 (s, 3H).

EXAMPLE 5

Synthesis of 4-hydroxy-7-methoxy-2(5-methyl-1,3,4-oxadiazol-2-yl) quinoline (5e)



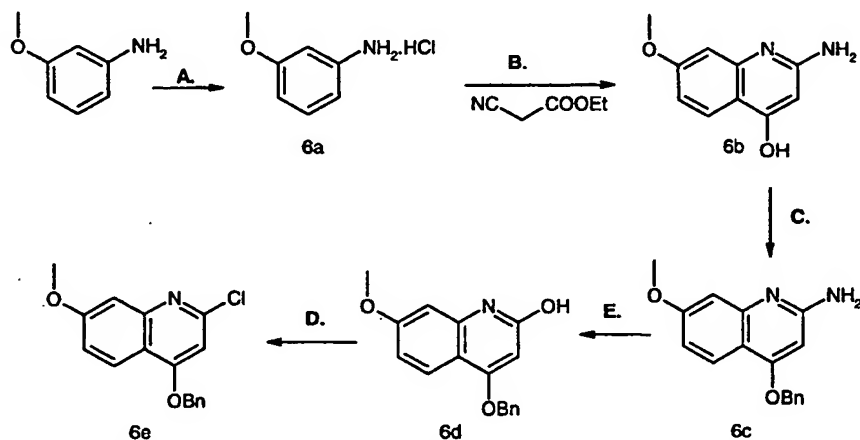
- 15 A. To substrate 4b (465 mg, 1.45 mmol) in ethanol (5 mL) was added anhydrous hydrazine (57 μL , 1.8 mmol). The resulting solution was heated at reflux for 4 h, then concentrated under vacuum to afford product 5a (704 mg, quantitative crude yield) as a yellow solid which was used as such in the next step.
- B. Compound 5a (assumed 1.45 mmol) in triethylorthoacetate (5 mL) was

heated at 100-110 °C under nitrogen. The resulting mixture was then diluted with EtOAc (100 mL), washed with aqueous saturated NaHCO_3 (50 mL), brine (50 mL), dried with MgSO_4 , concentrated under vacuum and purified by flash column chromatography (100% EtOAc). Compound 5b (359 mg, 61% yield for two steps) was obtained as a yellow oil. MS (ES+) 392 (m+1), (ES-) 390 (m-1).

C. Compound 5b (333 mg, 0.852 mmol) was heated at 140 °C under high vacuum for 8.5 h and purified by flash column chromatography (100% EtOAc) to afford a mixture of 5b (116 mg, 35%, R_f 0.5) and compound 5c (138 mg, 72% corrected yield, R_f 0.3). To a THF (4 mL) solution of compound 5c (138 mg, 0.4 mmol) was added aqueous HCl (1 N, 1 mL) and the resulting mixture was stirred until completion (30 min.). THF was evaporated under vacuum and aqueous NaH_2PO_4 (1 M, 2 mL) was added. The resulting suspension was sonicated, filtered and the solid was dried under high vacuum to afford the desired product 5d, (75 mg, 73% yield) as a beige solid. MS (ES+) 258 (m+1), (ES-) 256 (m-1). ^1H NMR (400 MHz, DMSO-d): δ 8.03 (d, J = 9.2 Hz, 1H), 7.39 (d, J = 2.2 Hz, 1H), 7.06 (br. d, J = 8.6 Hz, 1H), 6.85 (br. s, 1H), 3.88 (s, 3H), 2.64 (s, 3H).

EXAMPLE 6

Synthesis of 4-benzyloxy-2-(chloro)-7-methoxyquinoline (6e)



A. Commercially available *Meta*-anisidine (25 g, 0.20 mol) in dioxane (80 mL) was cooled down to 0 °C and anhydrous HCl (4 N/dioxane, 75 mL, 0.30 mol) was added. Then Et₂O (500 mL) was added and stirring was maintained for 1 hour. The

beige solid was then filtered and dried under vacuum to afford salt 6a (31.88 g, 98% yield).

B. To this salt was added ethylcyanoacetate (21.3 mL, 0.20 mol) and the mixture, in a flask equipped with a distillation head and a collecting flask, was heated to 280–300 °C. Ethanol produced was collected to monitor the evolution of the reaction. At 9 mL of collected ethanol (theoretical amount 11.7 mL), heating was stopped, the reaction mixture cooled down to RT, diluted with water (200 mL) – EtOAc (200 mL) then stirred and aqueous NaH_2PO_4 (300 mL) was added. After additional stirring for 1 h, filtration and drying, 6b was obtained (19.06 g, 84.5% purity, ~50% yield) as a yellow solid and was used as such in the next reaction.

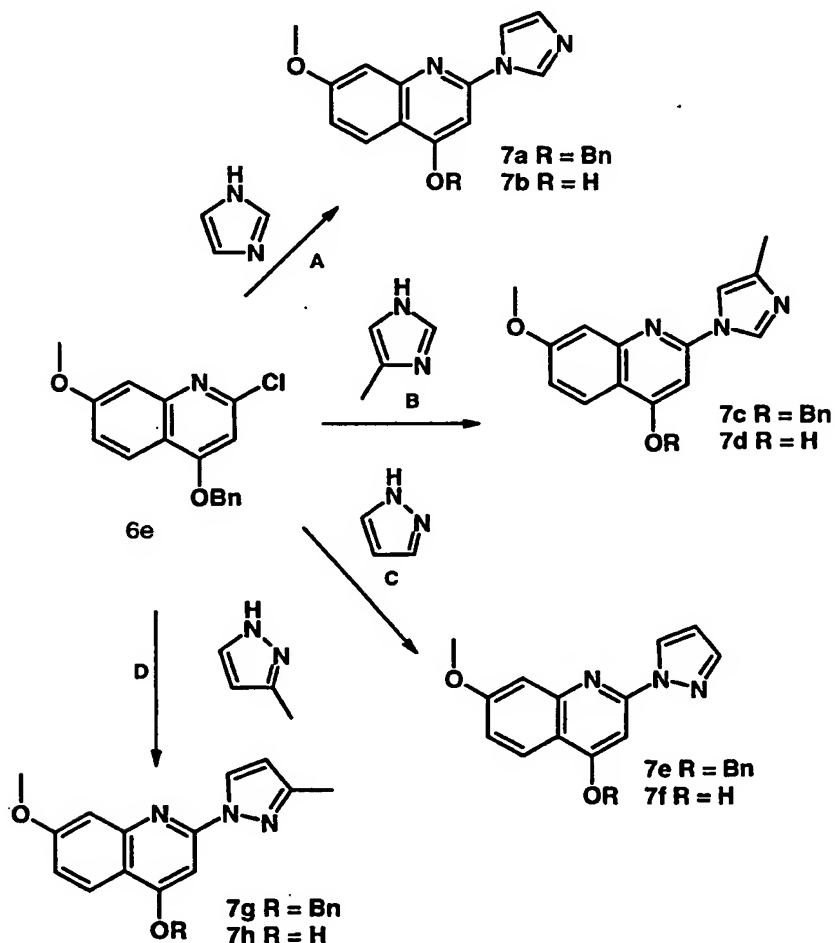
C. Compound 6b (11.0 g, 57.8 mmol) in DMF (100 mL) at 0 °C was added to NaH (60% in mineral oil, 2.78 g, 115.6 mmol). The ice bath was then removed and the mixture was stirred at ambient temperature for 1 h, benzyl bromide (7.6 mL, 63.6 mmol) was then added and the reaction mixture was stirred for 16 hours. The solution was then diluted with EtOAc (220 mL) – hexane (220 mL) and the solid formed was filtered, triturated with aqueous saturated NaHCO_3 (110 mL), washed with water, hexane–EtOAc (1:1 ratio, 100 mL) and dried under high vacuum. Product 6c (5.6 g, 91% purity, 35% yield) was thus obtained as a yellow solid. To compound 6c (2.67 g, 9.52 mmol) in acetic acid (21 mL) was added *iso*-amyl nitrite (3.8 mL, 28.6 mmol) and the resulting mixture was stirred at ambient temperature and monitored by HPLC. More *iso*-amyl nitrite (1.3 mL, 9.52 mmol) was added after 2 hours and the mixture was left to stir over 90 hours (HPLC 81% product, 3% substrate). Water (100 mL) was added to the resulting suspension, which was then filtered. The brown solid collected was dried under high vacuum giving product 6d (2.35 g, 92% purity, 72% yield).

D. To compound 6d (1.5 g, 4.39 mmol) was added phosphorous oxychloride (13 mL, 141 mmol) and the resulting mixture was heated at reflux for 1 hour then diluted with EtOAc (150 mL) and quenched at 0 °C slowly with aqueous NaOH (1 N, 150 mL) to pH 9. The two layers were separated and the organic layer was dried with MgSO_4 and concentrated under vacuum to afford a brown solid which was purified by flash column chromatography (15 % EtOAc/hexane). Product 6e (819 mg, purity > 99%, 62% yield) was obtained as a yellow solid.

^1H NMR (400 MHz, CDCl_3): δ 8.07 (d, $J = 9.2$ Hz, 1H), 7.50-7.40 (m, 5H), 7.29 (d, $J = 2.5$ Hz, 1H), 7.12 (dd, $J = 9.2, 2.5$ Hz, 1H), 6.73 (s, 1H), 5.26 (s, 2H), 3.92 (s, 3H).

EXAMPLE 7

Synthesis of 4-hydroxy-2-(1-imidazolyl)-7-methoxyquinoline (7b); 4-hydroxy-2-(4-methyl-1-imidazolyl)-7-methoxyquinoline (7d); 4-hydroxy-7-methoxy-2-(1-pyrazolyl)quinoline (7f); and 4-hydroxy-2-(3-methyl-1-pyrazolyl)-7-methoxyquinoline (7h).



A. Compound 6e (423 mg, 1.41 mmol) and imidazole (400 mg, 5.88 mmol.) were heated at 110 °C for 20 h. The mixture was then diluted with EtOAc and washed with water and brine, dried with MgSO_4 , concentrated under reduced pressure to afford compound 7a (422 mg, 96% purity, 90% yield) as a yellow solid. Compound 7a (319 mg, 0.963 mmol) with Pd (5%/C, 64 mg) in a mixture of ethanol (5 mL) and

THF (5 mL) was purged and placed under one ATM. of hydrogen. After 7.5 h of stirring at ambient temperature, the reaction mixture was filtered, rinsed with a chloroform-methanol mixture, and concentrated to afford **7b** (130 mg, 97.7% purity, 56% yield) as a yellow solid. MS (ES+) 242 (m+1), (ES-) 240 (m-1).

- 5 ^1H NMR (400 MHz, DMSO-d): δ 8.51 (s, 1H), 8.03 (d, J = 8.9 Hz, 1H), 7.93 (s, 1H), 7.23 (d, J = 1.9 Hz, 1H), 7.15 (s, 1H), 7.12 (dd, J = 9.2, 2.2 Hz, 1H), 6.92 (br. s, 1H), 3.91 (s, 3H).

- B. Compound **6e** (251 mg, 0.837 mmol) and 4-methylimidazole (344 mg, 4.19 mmol.) were heated at 110 °C for 20 h. The mixture was then diluted with EtOAc, washed with water and brine, dried with MgSO_4 , and concentrated under reduced pressure to afford a crude containing a 10:1 mixture of 4-methyl and 5-methylimidazolyl isomer respectively. The major assumed desired isomer **11c**, a white solid, (166 mg, 99% purity, 57% yield) was separated from a second more polar fraction (76 mg, 23% yield) containing a mixture of 4- and 5-methyl imidazolyl isomer by flash column chromatography (100% EtOAc). Compound **7c** (163 mg, 0.472 mmol) with Pd (5%/C, 33 mg) in a mixture of ethanol (2.4 mL) and THF (5 mL) was purged and placed under one ATM. of hydrogen. After 18 h of stirring at ambient temperature, the reaction mixture was filtered, rinsed with a chloroform-methanol mixture, and concentrated to afford **7d** (118 mg, 99% purity, 98% yield) as a white solid.

^1H NMR (400 MHz, DMSO-d): δ 8.42 (br. s, 1H), 8.01 (d, J = 9.2 Hz, 1H), 7.64 (br. s, 1H), 7.21 (br. s, 1H), 7.10 (d, J = 8.9 Hz, 1H), 6.89 (br. s, 1H), 3.90 (s, 3H), 2.20 (s, 3H).

- C. Compound **6e** (184 mg, 0.614 mmol) and pyrazole (209 mg, 3.07 mmol.) were heated at 110 °C for 17 h. The mixture was then diluted with EtOAc and washed with aqueous NaOH (1 N) and brine, dried with MgSO_4 , concentrated under reduced pressure to afford a crude product which was purified by flash column chromatography (2 : 1 hexane-EtOAc) to afford **7e** (103 mg, 50% yield) as a pale yellow solid. Compound **7e** (103 mg, 0.311 mmol) with Pd (5%/C, 20 mg) in a mixture of ethanol (2 mL) and THF (2 mL) was purged and placed under one atm. of hydrogen. After 5.5 h of stirring at ambient temperature, the reaction mixture was filtered, rinsed with a chloroform-methanol mixture, and concentrated to afford **7f**

43

(77 mg, 99% purity, 99% yield) as a yellow solid. MS (ES+) 242 (m+1), (ES-) 240 (m-1).

¹H NMR (400 MHz, DMSO-d): δ 8.72 (d, J = 2.5 Hz, 1H), 8.31 (s, 1H), 8.00 (d, J = 8.9 Hz, 1H), 7.83 (br. s, 1H), 7.43 (br. s, 1H), 7.24 (br. s, 1H), 7.10 (d, J = 8.6 Hz, 1H), 6.59 (br. s, 1H), 3.90 (s, 3H).

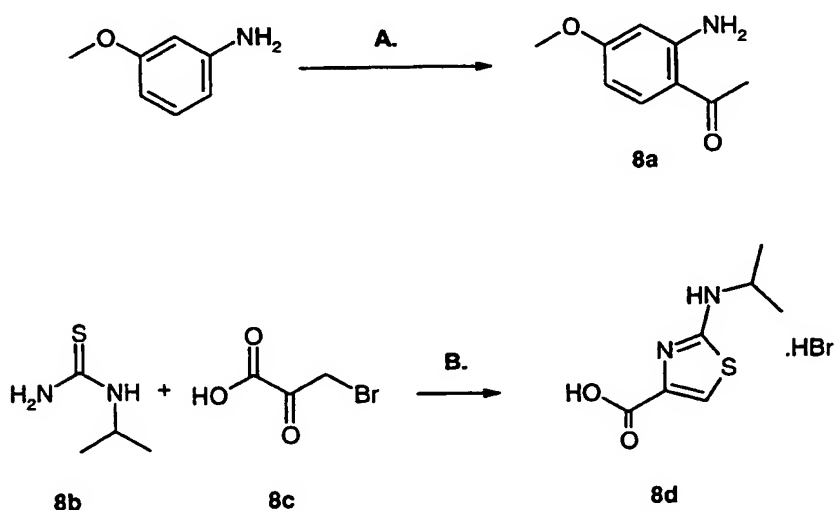
D. Compound 6e (217 mg, 0.724 mmol) and 4-methypyrazole (594 mg, 7.24 mmol.) were heated at 110 °C for 23 h. The mixture showing a 1 : 1 mixture of debenzylated compound 7h and benzylated product 7g was then diluted with EtOAc (2-3 mL) and filtered to afford the pure debenzylated product 7h (111 mg, 95% purity, 54% yield) as a white solid.

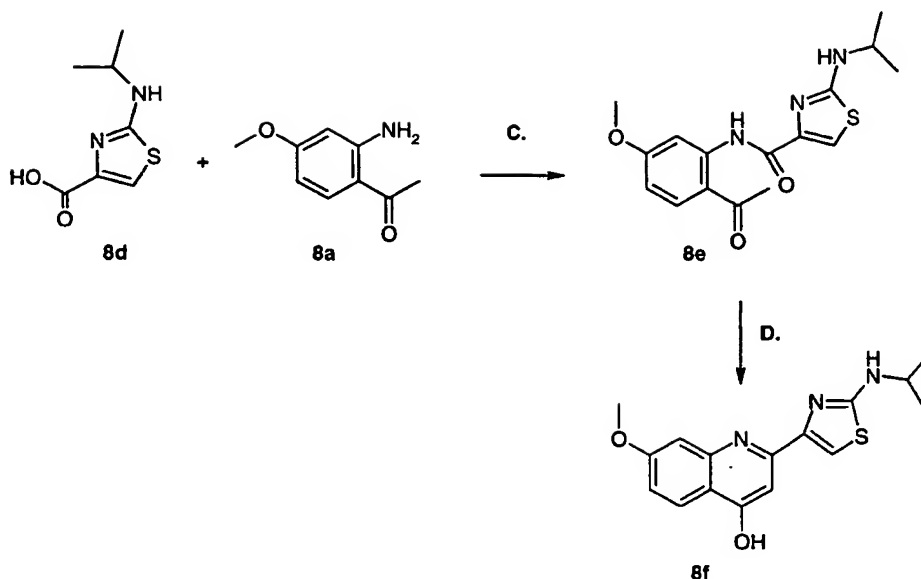
¹H NMR (400 MHz, DMSO-d): δ 8.58 (d, J = 2.6 Hz, 1H), 7.98 (d, J = 9.2 Hz, 1H), 7.25 (br. s, 1H), 7.20 (s, 1H), 7.04 (br. d, J = 9.2 Hz, 1H), 6.38 (s, 1H), 3.89 (s, 3H), 2.30 (s, 3H).

EXAMPLE 8

15 Synthesis of 4-hydroxy-7-methoxy-2[4(2-isopropylaminothiazolyl)] quinoline (8f)

Note: [A variety of 2-alkylaminothiazolyl substituents were made using the same synthetic scheme where compound 8b was replaced by other alkyl thioureas.]





- A. The protocol used for the conversion of *m*-anisidine to 8a was identical to that described in the literature: F.J. Brown *et al.* *J. Med. Chem.* **1989**, 32, 807-826. However, the purification procedure was modified to avoid purification by chromatography. The EtOAc phase containing the desired product was treated with a mixture of MgSO₄, charcoal and 5% w/w (based on expected mass) silica gel. After filtration on celite, the product was triturated with ether. Compound 8a was obtained as a pale brown solid in >99% purity (as confirmed by HPLC).
- B. A suspension of isopropyl thiourea (8b, 3.55 g, 30 mmol) and 3-bromopyruvic acid (8c, 5 g, 1 eq.) in dioxane (300 mL, 0.1 M) was heated to 80 °C. Upon reaching 80 °C the solution became clear and soon after the product precipitated as a white solid. After 2 hours of heating, the solution was cooled to RT and the white precipitate was filtered to obtain compound 8d in high purity (>98% purity as confirmed by NMR) and 94% yield (7.51 g).
- C. A mixture of the carboxylic acid 8d (4.85 g, 18.2 mmol) and the aniline derivative 8a (3 g, 1eq.) in pyridine (150 mL, 0.12 M) was cooled to -30 °C (upon cooling, the clear solution became partially a suspension). Phosphorus oxychloride (3.56 mL, 2.1 eq.) was then added slowly over a 5 min period. The reaction was stirred at -30 °C for 1 h, the bath was removed and the reaction mixture was allowed to warm-up to RT. After 1.5 h the reaction mixture was poured into ice, the pH was

adjusted to 11 with aqueous 3N NaOH, extracted with CH_2Cl_2 , dried over anhydrous MgSO_4 , filtered and concentrated under vacuum. The beige solid was then purified by flash chromatography (45% EtOAc in hexane) to give compound 8e as a pale yellow solid in 73% yield (6.07 g).

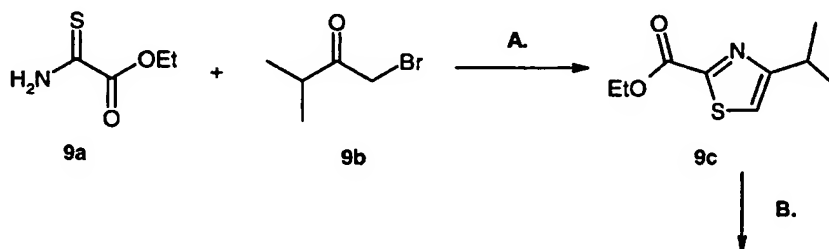
- 5 D. A solution of tBuOK (2.42 g, 21.6 mmol) in anhydrous tBuOH (40ml, 0.14 M, distilled from Mg metal) was heated to reflux. Compound 8e (1.8g, 5.4 mmol) was added portion-wise over 5 min and the dark red solution formed was stirred at reflux for an additional 20 min (completion of the reaction was monitored by HPLC). The mixture was cooled to RT and HCl was added (4 N in dioxane, 1.5 eq.).
- 10 The mixture was then concentrated under vacuum, in order to assure that all of the HCl and dioxane were removed, the product was re-dissolved twice in CH_2Cl_2 and dried under vacuum to finally obtain the HCl salt of compound 8f as a beige solid (1.62 g, 93% pure by HPLC). The product was then poured into a phosphate buffer (1N NaH_2PO_4 , pH \approx 4.5) and sonicated. The beige solid was filtered and dried under
- 15 vacuum to give compound 8f (1.38 g, 81% yield) as a beige solid (91% pure by HPLC).

^1H NMR (400 MHz, DMSO) δ 8.27 (s, 1H), 8.12 (d, 1H, $J = 9.2$ Hz), 7.97 (br.s, 1H), 7.94 (s, 1H), 7.43 (s, 1H), 7.24 (dd, 1H, $J = 9.2, 2.2$ Hz), 3.97 (m, 1H), 3.94 (s, 3H), 1.24 (d, 2H, $J = 6.4$ Hz).

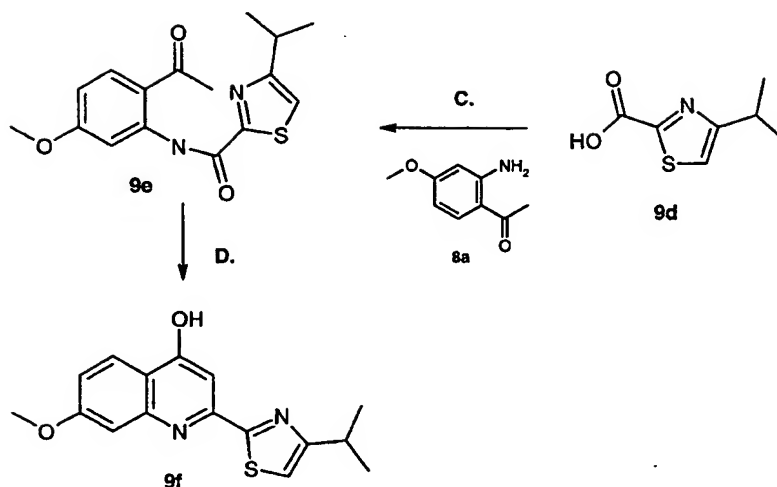
20 **EXAMPLE 9**

Synthesis of 4-hydroxy-7-methoxy-2[2(4-isopropylthiazolyl)]quinoline (9f).

Note: A variety of 2-(4-alkyl)-thiazolyl substituents were made using the same synthetic scheme where compound 9b was replaced by other α -bromoketones.



46



- A. To a solution of 3-methyl-butan-2-one (8 g, 93 mmol) in MeOH (100 mL) at –30 °C, Br₂ (4.79 mL, 93 mmol, 1 eq.) was added dropwise over a period of 45 min. The resulting mixture was then stirred at RT for 90 min. Pentane was added and the solution washed with 5% aqueous NaHCO₃, the organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under vacuum. The resulting crude yellow oil, compound **9b**, was used without further purification. A solution of ethyl thiooxamate (**9a**, 1.8 g, 13.5 mmol) and bromoketone derivative **9b** (13.5 mmol.) was stirred at 70 °C for 15 h. The mixture was then concentrated under vacuum and subsequently purified by flash column chromatography, using 15% EtOAc in hexane as the eluent, to obtain compound **9c** (740 mg, 28 % yield).
- B. A solution of compound **9c** (700 mg, 3.5 mmol) in THF/MeOH/H₂O (3:1:1 ratio, 13 mL) was treated with LiOH·H₂O (148 mg, 3.5 mmol, 1eq.) at RT for 5 h. The pH was then adjusted to 6 with 0.1N HCl and the mixture was concentrated to dryness under vacuum to obtain the acid **13d**, which was used directly in the next step without further purification.
- C. A solution of 4-methoxy-2-aminoacetophenone (intermediate **8a**, 570 mg, 3.45 mmol) and carboxylic acid derivative **9d** (590 mg, 3.45 mmol, 1eq.) in pyridine (30 mL) was cooled to –20 °C. POCl₃ (0.35 mL, 3.79 mmol, 1.1 eq.) was then added dropwise over a period of 5 min. The resulting solution was stirred at –10° C for 2 h. The reaction was quenched with the addition of H₂O and the mixture was

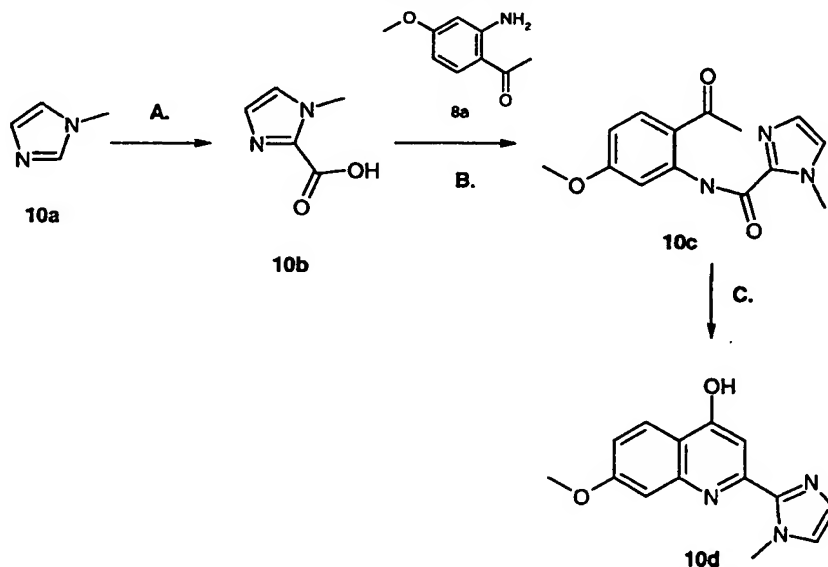
concentrated under vacuum. The residue was poured in a saturated aqueous solution of NaHCO_3 and extracted with EtOAc. The organic layer was dried over anhydrous MgSO_4 , filtered and concentrated under vacuum. The crude product was purified by flash column chromatography, using 25%EtOAc in hexane as the eluent, to give compound 9e as a white solid (740 mg, 67% yield).

D. tBuOK (518 mg, 2.1 eq.) was added to a suspension of compound 9e (700 mg, 2.2 mmol) in anhydrous tBuOH (11 mL). The resulting mixture was heated to 75°C for 7.5 h, the solution was then cooled to RT and acidified with the addition of HCl (4N HCl in dioxane, 2.5 mL). The mixture was concentrated under vacuum and the residue obtained was poured into a solution of 1N NaH_2PO_4 and filtered. The solid material was then triturated with a small amount of EtOAc, filtered and dried under vacuum to obtain compound 9f as a pale beige solid (270 mg, 41% yield).

^1H NMR (400 MHz, DMSO-d_6) δ 8.00 (br. s, 1H), 7.60 (br. s, 1H), 7.51 (br. s, 1H), 7.43 (br. s, 1H), 7.29 (br. s, 1H), 7.14 (br. s, 1H), 6.95 (br. a, 1H), 3.90 (s, 3H), 3.15 (m, 1H), 1.33 (d, $J=5.4$ Hz, 6H).

EXAMPLE 10

Synthesis of 4-hydroxy-2-(1-methyl-2-imidazolyl)-7-methoxyquinoline (10d)

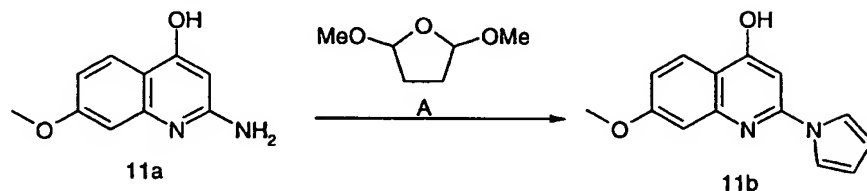


A. A solution of N-methylimidazole 10a (5g, 61 mmol) in 100 mL THF was cooled at -78°C . $n\text{-BuLi}$ (24.4 ml of a 2.5M/ Et_2O solution, 1 eq.) was added

dropwise over 15 min. The resulting mixture was stirred 90 min. at -78° C then poured portionwise over excess solid CO₂. The heterogeneous mixture was stirred 2 h and allowed to reach RT. 1N HCl was added to pH 5, the aqueous layer is separated and lyophilized. The residue thus obtained was extracted with EtOAc (to
5 remove salts), dried (Na₂SO₄), filtered and concentrated under reduced pressure. 6.2 g (80% yield) of a white solid **10b** was obtained.

B. A solution of 4-methoxy-2-amino-acetophenone **8a** (394 mg, 2.39 mmol) and the carboxylic acid derivative **10b** (301 mg, 1eq.) in pyridine (10 ml) was cooled to -20° C. POCl₃ (244 µl, 1.1 eq.) was then added dropwise over 5 min.. The resulting
10 solution was stirred at -10 °C for 2.5 h. Water was then added and the mixture was concentrated under reduced pressure. The residue was poured in a saturated solution of NaHCO₃ and extracted with EtOAc. The organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. The product was purified by chromatography using silica gel (25% EtOAc/Hex) affording 530 mg
15 (81% yield) of a pale yellow solid **10c**.

C. tBuOK (431 mg 2.1 eq.) was added to a suspension of the substrate **10c** (500 mg, 1.8 mmol) in 8 ml of tBuOH. The resulting mixture was then heated to 75°C for 7 h. The solution was allowed to reach room temperature overnight and 2.5 ml of HCl (4N/dioxane) was added. The mixture was concentrated under reduced
20 pressure and the residue obtained was diluted with EtOAc. NaOH 1N was added until a pH of 7 was obtained. The organic phase was separated and dried (MgSO₄), filtered, and concentrated under reduce pressure to afford 145 mg of **10d** (31% yield) as a pale beige solid. ¹H NMR (400 MHz, DMSO-d): δ 7.99 (d, J = 8.9 Hz, 1H), 7.49 (s, 1H), 7.37 (s, 1H), 7.18 (s, 1H), 6.92 (d, J = 8.9 Hz, 1H), 6.31 (s, 1H), 3.87 (s, 3H), 3.84 (s,
25 3H).

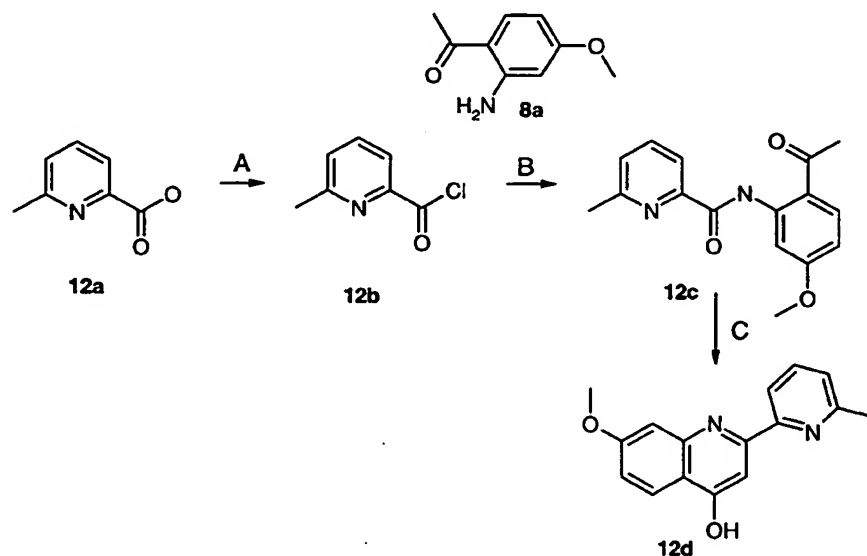
EXAMPLE 11**Synthesis of 4-hydroxy-2-(1-pyrrolyl)-7-methoxyquinoline (11b)**

- A. A solution of the substrate 11a (obtained from compound 6c after
 5 hydrogenolysis of the benzyl group with 5% Pd/C in ethanol-THF) (1g, 5.25 mmol)
 and 2,5-dimethoxytetrahydrofuran (0.68 ml, 1 eq.) in glacial acetic acid was refluxed
 for 4.5 h and allowed to reach RT. The mixture was then concentrated under
 reduced pressure. The residue was diluted with methanol and NaOH(aq.) 1N was
 added until pH 7 is reached. The product was purified by chromatography using
 10 silica gel (3%MeOH/CH₂Cl₂, the residue was pre-adsorbed on silica gel). 140 mg
 (13% yield) of 11b as a white solid was obtained.

¹H NMR (400 MHz, DMSO-d): δ 7.98 (d, J = 9.2 Hz, 1H), 7.64 (s, 2H), 7.18 (d, J = 2.5 Hz, 1H), 7.05 (br. d, J = 7.9 Hz, 1H), 6.88 (br. s, 1H), 6.32 (s, 2H), 3.90 (s, 3H).

EXAMPLE 12

- 15 **Synthesis of 4-hydroxy-7-methoxy-2-(6-methyl-2-pyridyl)quinoline (12d)**



- A. 6-Methylpicolinic acid 12a (411 mg, 3.0 mmol) and SOCl₂ (0.520 mL, 7.2 mmol, 2.4 eq.) were refluxed in benzene (5 mL) for 2 h. The solvent and excess

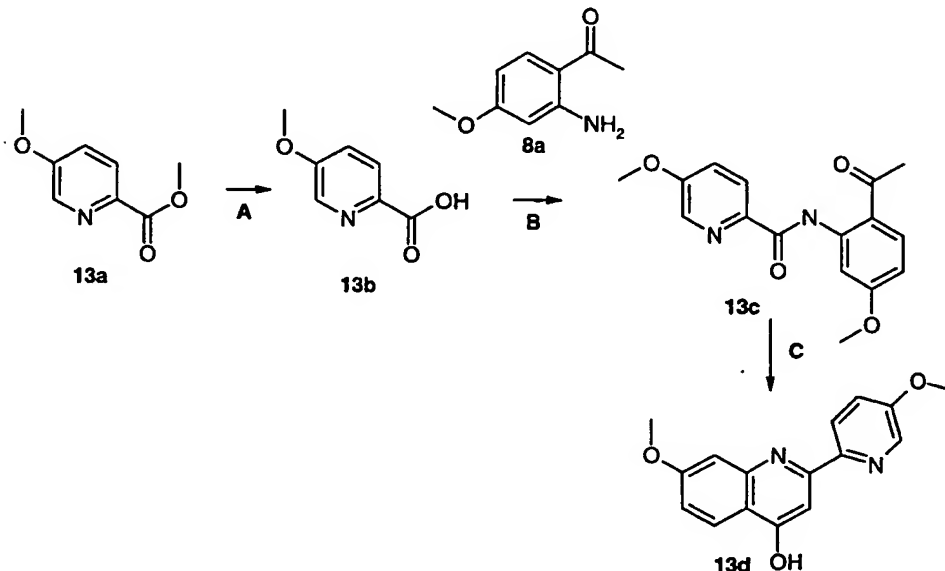
SOCl_2 were removed from the reaction mixture under vacuum and the residue was triturated with pentane. The solid material formed was filtered off and the filtrate concentrated to give the acid chloride **12b** (500 mg, 2.6 mmol).

B. To a solution of the crude acid chloride **12b** in CH_2Cl_2 (5 mL) at 0°C , a
5 solution of the aniline **8a** (344 mg, 2.08 mmol), DIPEA (1.45 mL, 8.35 mmol) and DMAP (61 mg, 0.5 mmol) in CH_2Cl_2 (10 mL) was added. The reaction mixture was stirred at RT for 16 h. The volatile components were removed under vacuum, the residue was dissolved in EtOAc and the solution was washed with 5% NaHCO_3 (2x), H_2O and brine. The organic layer was then dried over MgSO_4 and concentrated
10 under vacuum. The mixture was purified by flash column chromatography, using EtOAc/ hexane (1:2) as the eluent, to obtain the amide **12c** (490 mg, 82%).

C. To a suspension of amide **12c** (490 mg, 1.71 mmol) in t-BuOH (10 mL), tBuOK (410 mg, 3.43 mmol) was added and the mixture was stirred at 75°C for 6 h and then at RT for 16 h. The mixture was then poured in phosphate buffer (175 mL, pH= 7) and stirred for 30 min. The solid was triturated twice with ethyl acetate.
15 The organic phase was washed with brine, dried over MgSO_4 and concentrated under vacuum. The solid obtained was triturated with EtOAc to give the quinoline derivative **12d** (263 mg, 58%). ^1H NMR: (CDCl_3 , 400 MHz): δ 2.68 (s, 3 H), 3.94 (s, 3 H), 6.85-6.88 (2d, J= 8.68 & 9.5 Hz, 2 H), 6.94 (dd, J = 8.9 & 2.2 Hz, 1 H), 7.27 (dd, J= 6.7 & 1.9 Hz, 1 H), 7.73-7.79 (m, 2 H), 8.28 (d, J = 8.9 Hz, 1 H), 10.3 (br s, 1 H).
20

EXAMPLE 13

Synthesis of 4-hydroxy-7-methoxy-2-(5-methoxy-2-pyridyl)quinoline (13d)



A. To a solution of compound 13a (623 mg, 3.73 mmol) in MeOH, NaOH (2M, 4.70 mL) was added and the reaction mixture was stirred at RT for 2 h. The solution was then acidified with HCl (6N, 2.2 mL) and concentrated to obtain compound 13b, which was used in the following step without purification.

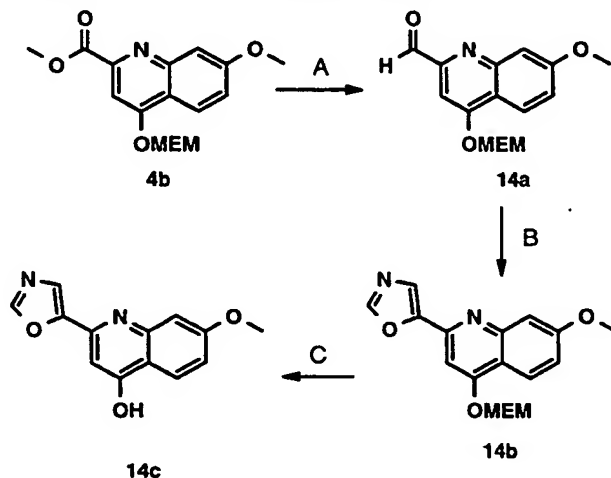
B. To a solution of the crude compound 13b (~3.73 mmol) in pyridine (25 mL), the aniline 8a (500 mg, 3.03 mmol) was added and the solution was cooled to -25°C before POCl_3 (0.35 mL, 3.73 mmol) was added. The reaction mixture was stirred at -10°C for 1 h and then at 0°C for 2 h. The mixture was then poured onto H_2O and extracted with EtOAc (2-3x). The combined organic layers were washed with 5% NaHCO_3 and brine, dried over MgSO_4 and concentrated under vacuum. The crude material was purified by flash column chromatography, using EtOAc/ hexane (1:2) as the eluent, to give the amide 13c (617 mg, 55%).

C. To a suspension of the amide 13c (617 mg, 2.05 mmol) in anhydrous t-BuOH (10 mL), tBuOK (490 mg, 4.11 mmol) was added and the mixture was stirred at 75°C for 6 h and then at RT for 16 h. The reaction mixture was poured in phosphate buffer (175 mL, pH= 7) and stirred for 30 min. The solid material formed was filtered and triturated with EtOAc to give the quinoline derivative 13d (250 mg,

43%). ^1H NMR: (DMSO, 400 MHz): δ 3.86 (s, 3 H), 3.94 (s, 3 H), 6.72 (bs, 1 H), 6.91 (dd, $J = 8.9$ & 1.9 Hz, 1 H), 7.54 (d, $J = 1.9$ Hz, 1 H), 7.60 (dd, $J = 8.9$ & 2.9 Hz, 1 H), 7.97 (d, $J = 8.9$ Hz, 1 H), 8.21 (d, $J = 8.6$ Hz, 1 H), 8.48 (d, $J = 1.9$ Hz, 1 H).

EXAMPLE 14

5 Synthesis of 4-hydroxy-7-methoxy-2-(oxazol-5-yl) quinoline (14c):



- A. The protected quinoline derivative 4b from Example 4 (3.8 g, 11.8 mmol) was dissolved in CH_2Cl_2 (60 mL) and cooled to -78°C before diisobutylaluminum hydride (7.9 mL, 1 equiv., 1.5M in toluene) was added very slowly over 15 min.
- 10 After stirring for 80 min, an additional amount of DIBAL was added (5.5 mL, 0.7 equiv., 1.5 M in toluene). After stirring at -78°C a further 2 h, the reaction was carefully quenched with methanol (4 mL) at -78°C and then poured into aqueous solution of Rochelle salt (1N K-Na tartrate). The thick paste was stirred with CH_2Cl_2 (300 mL) for 2 h until clear. The phases were separated and the organic phase dried
- 15 (MgSO_4), filtered and concentrated to give a white solid. Purification by flash chromatography (SiO_2 , 230-400 mesh) with 50% EtOAc /hexane gave aldehyde 14a as a white solid (2.5g, 73%).

- B. To a stirred suspension of K_2CO_3 (48 mg, 0.34 mmol) in MeOH (7 mL) was added toluenesulphonylmethylisocyanide (66 mg, 0.34 mmol). The reaction was
- 20 heated to 45°C and aldehyde 14a (0.10 g, 0.34 mmol) was added. The reaction mixture was heated to 80°C for 16 h and then concentrated to dryness under

vacuum. Purification was performed by flash chromatography (SiO_2 , 230-400 mesh) to afford the desired oxazole **14b** (0.089 g, 80%). MS: 331.0 ($\text{M} + \text{H}$)⁺.

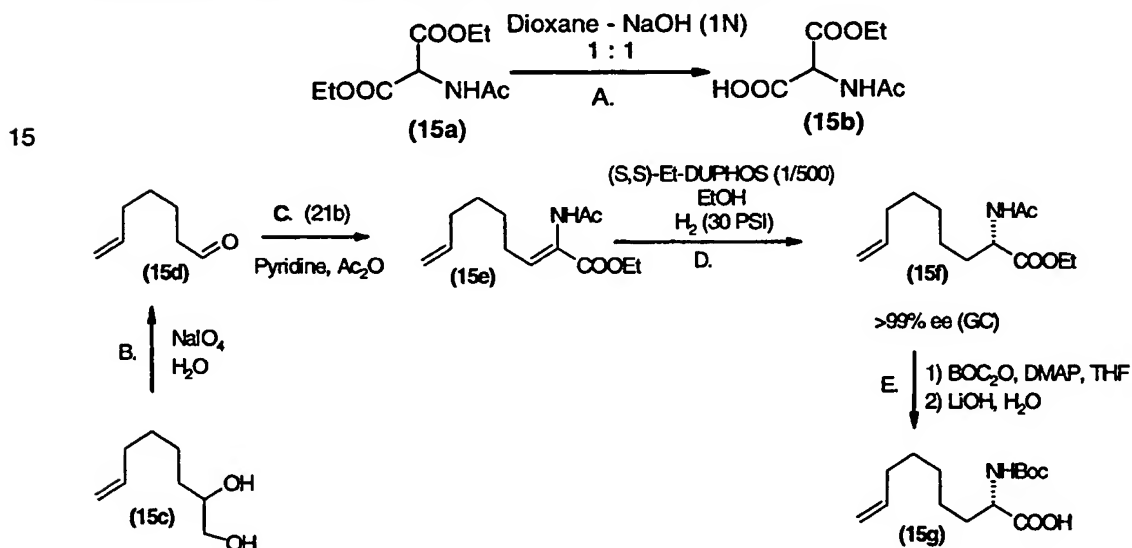
C. The MEM protected hydroxyquinoline **14b** was dissolved in THF (3 mL) and treated with aqueous HCl (1N, 1 mL). The reaction was stirred for 30 min at RT before being concentrated to dryness under vacuum. The residue was treated with phosphate buffer (3 mL, 1 N solution, pH 4.5) and stirred before the product was filtered out, washed with distilled water and dried overnight under high vacuum (60 °C, 16 h). The desired hydroxy quinoline **14c** was obtained as a tan colored solid (0.065 g, 100%). MS: 242.9 ($\text{M} + \text{H}$)⁺.

¹H NMR ($\text{DMSO}-d_6$): δ 8.65 (s, 1H), 8.02 (bs, 1H), 7.97 (d, $J = 8.9$ Hz, 1H), 7.19 (s, 1H), 6.93 (d, $J = 7.9$ Hz, 1H), 6.42 (bs, 1H), 3.87 (s, 3H). ES (+) MS: m/z 242.9 ($\text{M} + \text{H}$)⁺.

PEPTIDE LINKER MOIETIES (P3)

EXAMPLE 15

Synthesis of (2S)-N-Boc-amino-non-8-enoic acid (**15g**)



A. To a solution of commercially available diethyl 2-acetamidomalonate **15a** (100 g, 0.46 mole) in dioxane (500 mL) was added aqueous sodium hydroxide (1 M, 1 eq., 460 mL) dropwise over 30 to 45 min. The resulting mixture was left to stir for 16.5 h, then dioxane was evaporated *in vacuo*, the aqueous solution was extracted with three portions of 300 mL of ethyl acetate and acidified to pH 1 with

20

concentrated HCl. This solution was left to crystallize in an ice-water bath. After the appearance of a few crystals, the mixture was sonicated and an abundant precipitate appeared. Filtration and drying under vacuum afforded compound 15b, (62.52 g, 72% yield) as a white solid.

5 B. To a magnetically stirred emulsion of commercially available 7-octene-1,2-diol 15c (25 g, 0.173 mole) and H₂O (100 mL), in a 1 L round bottom flask, an aqueous solution of sodium periodate (40.7 g, 0.190 moles, 1.1 eq., in 475 mL H₂O) was added over a period of 20 min (slightly exothermic). The resulting mixture was stirred at room temperature for an additional 1 h (completion of reaction confirmed
10 by TLC). The mixture was then decanted in a separatory funnel and the aqueous layer was separated from the organic layer. The aqueous solution was saturated with NaCl, decanted and separated from the organic fraction once more. The two organic fractions were combined, dried with sodium sulfate and filtered over a cotton plug (in a Pasteur pipette) to give compound 15d (15.135 g, colorless oil, 78%
15 yield). The aqueous solution was extracted with CH₂Cl₂, dried with anhydrous MgSO₄, and concentrated under vacuum (without heating, heptanal b.p.153°C) to obtain an additional amount of compound 15d (1.957 g, colorless oil, 10% yield). Total yield 88%.

C. To solid ethyl 2-acetamidomalonate 15b (7.57 g, 40 mmol.) was added 6-
20 heptenal 15d (4.48 g, 40 mmol) in solution in pyridine (32 mL, 10 eq) over 1 min. The resulting solution was cooled in a 10° C bath and acetic anhydride (12 mL, 3.2 eq.) was added over 4 min. The resulting orange solution was stirred for 3 h at RT and another portion of ethyl 2-acetamidomalonate 15b (2.27 g) was added. The resulting mixture was stirred at room temperature for an extra 11 h. Ice (60 mL) was
25 then added and the solution was stirred for 1.5 h, then the mixture was diluted with 250 mL of water and extracted with two portions of ether. The ethereal solution was washed with 1N HCl, sat. NaHCO₃, dried Na₂SO₄, concentrated and purified by flash chromatography (EtOAc 40% / hexane) to give compound 15e (4.8g, 50% yield) as a pale yellow oil.

30 D. To a degassed (argon bubbling for 30 min.) solution of Z-ethyl 2-acetamido-2,8-nonadienoate 15e (8.38g, 35 mmol) in dry ethanol (70 mL) was added (S,S)-Et-

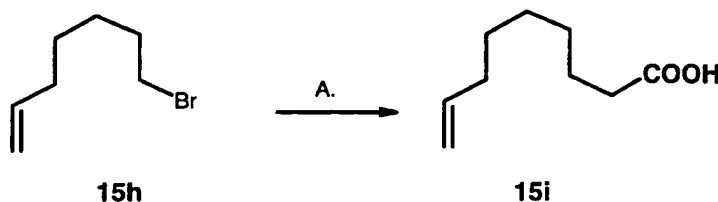
55

DUPHOS Rh(COD)OTf (51 mg, S/C = 496). The mixture was put under 30 psi of hydrogen (after 4 vacuum-H₂ cycles) and stirred on a Parr shaker for 2 h. The resulting mixture was evaporated to dryness to obtain the crude compound 15f, which was used in the subsequent step without purification.

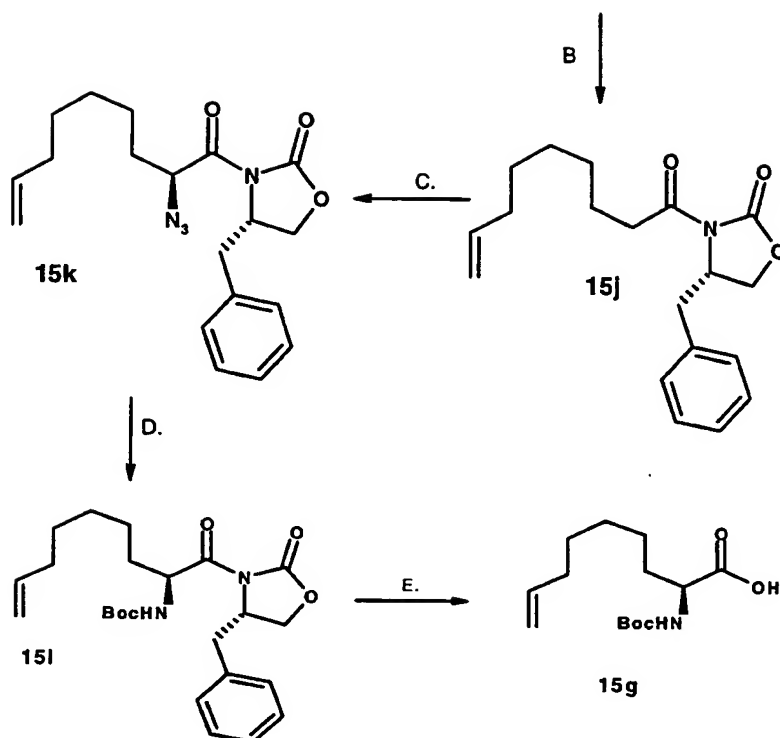
- 5 E. To a solution of crude (*S*)-ethyl 2-acetamido-8-nonenoate 15f (7.3 g, 30.3 mmol) in THF (100 mL), Boc₂O (13.2 g, 2 eq.) and DMAP (740 mg, 0.2 eq) were added, and the reaction mixture was heated at reflux for 2.5 h. Subsequently, most of the THF solvent was evaporated, the crude mixture was diluted with CH₂Cl₂ and washed with 1 N HCl in order to remove the DMAP. The organic layer was further
- 10 extracted with saturated aqueous NaHCO₃, dried with anhydrous Na₂SO₄ and concentrated under vacuum. The crude product was then diluted with THF (50 mL) and water (30 mL), LiOH.H₂O (2.54 g, 2 eq.) was added and the resulting mixture was stirred at RT for 25 h (completion of the hydrolysis was confirmed by TLC). The reaction mixture was concentrated under vacuum to remove most of the THF
- 15 solvent and diluted with CH₂Cl₂. The resulting solution was washed with 1 N HCl, dried with anhydrous Na₂SO₄ and concentrated under vacuum. In order to remove minor impurities and excess Boc₂O, the crude product was purified by flash chromatography (using a solvent gradient from 100% hexane – 100% EtOAc as the eluent). The titled compound 15g was obtained in high purity as a pale yellow oil
- 20 (5.82 g, 71% yield). ¹H NMR (DMSO, 400 MHz): δ 7.01 (d, J = 8 Hz, 1H), 5.79 (tdd, J_t = 6.7 Hz, J_d = 17.0, 10.2 Hz, 1H), 5.00 (md, J_d = 17.0 Hz, 1H), 4.93 (md, J_d = 10.2 Hz, 1H), 3.83 (m, 1H), 2.00 (q, J = 6.9 Hz, 2H), 1.65-1.5 (m, 2H), 1.38 (s, 9H), 1.35-1.21 (m, 6H).

EXAMPLE 15A

- 25 Alternative synthesis of (2*S*)-N-Boc-amino non-8-enoic acid (15g):



56



- A. To a stirred suspension of finely cut Mg ribbons (0.55 g, 22.5 mmol) in dry THF (30 mL) containing dibromoethane (0.1 mL), 8-bromo-1-octene (15h, 2.52 mL, 15 mmol) was added dropwise over a period of 15 min, [the reaction is slightly exothermic]. After 30 min, the mixture was heated to 38° for 1 h and then cooled to -78° before it was added via a cannula onto an excess amount of solid CO₂. The mixture was diluted with diethyl ether (100 mL) and the solution was washed with brine (2 x 50 mL), dried over MgSO₄ and evaporated. A crude oil was obtained which was purified by chromatography on silica gel using 15% EtOAc in hexanes as the eluent to give compound 15i in 62% yield (1.44 g).

¹H NMR (CDCl₃, 400 MHz) δ 1.31-1.42 (m, 6H), 1.60-1.69 (m, 2H), 2.02-2.09 (m, 2H), 2.35 (t, J = 8.3 Hz, 2H), 4.99 (dm, J = 10.0 Hz, 1H), 5.04 (dm, J = 17.0 Hz, 1H), 5.75-5.86 (m, 1H).

- B. To a vigorously stirring solution of the carboxylic acid 15i (1.36g, 8.7 mmol) in anhydrous THF (70 mL) at -78°, freshly distilled Et₃N (1.6 mL; 11.3 mmol) and pivaloyl chloride (1.18 mL, 9.58 mmol) were added via a syringe under anhydrous conditions. The mixture was stirred at -78° for 15 min and then at 0° for 45 min.

The mixture was cooled again to -78° and then transferred via a cannula into an anhydrous solution of 4(S)-4-(phenylmethyl)-2-oxazolidinone lithium salt in THF at -78° ; the lithium salt of the oxazolidinone reagent had been previously prepared by the slow addition of *n*-BuLi (2.00 M in hexanes, 7.85 mL, 15.7 mmol) into a THF (20 mL) solution of the oxazolidinone (2.78g, 15.7 mmol) in THF at -78° .

The reaction mixture was stirred at -78° for 15 min then at RT for 1.5 h. Finally, it was quenched with an aqueous solution of sodium bisulfate (100 mL of 1 M) and the THF evaporated to $\frac{3}{4}$ of its initial volume. The residue was extracted with EtOAc (2 x 150 mL) and the mixed organic layers were washed with 5% NaHCO_3 (3 x 50 mL), brined (2 x 50 mL), dried over MgSO_4 and evaporated. The resulting crude oil was chromatographed on silica gel, using 15% EtOAc in Hexanes to obtain compound 15j in 68% yield (1.88g).

^1H NMR (CDCl_3 , 400 MHz) δ 1.35-1.47 (m, 6H), 1.67-1.74 (m, 2H), 2.02-2.09 (m, 2H), 2.65 (dd, $J = 13.4$ & 9.9 Hz, 1H), 2.84-3.02 (m, 2H), 3.31 (dd, $J = 13.4$ & 3.2 Hz, 1H), 4.13-4.22 (m, 2H), 4.62-4.71 (m, 1H), 4.93 (d, $J = 10.2$ Hz, 1H), 5.00 (dd, $J = 17.2$ & 1.6 Hz, 1H), 5.75-5.84 (m, 1H), 7.18-7.38 (m, 5H).

C. To a stirred solution of KHMDS (0.8 M THF, 22 mL, 17.5 mmol) in dry THF (50 mL) at -78° was cannulated a solution of the acid derivative 15j (3.25g, 10.30 mmol) in dry THF (40 mL) at -78° . The mixture was stirred at -78° for 45 min. To this mixture, a solution of trizylazide (3.67g, 11.85 mmol) in dry THF (40 mL) at -78° was added. The mixture was stirred at -78° for 3 min then quenched with acetic acid (5 mL). Subsequently, it was stirred at RT for 1 h and 45 min and finally at 40° for 15 min. Most of the THF was evaporated. The residue was taken into EtOAc (100 mL) and the organic solution washed with H_2O (50 mL), 5% NaHCO_3 (3x 50 mL) and brine (50 mL), (MgSO_4) and evaporated. The oil obtained was chromatographed on silica gel using Hexane/ CH_2Cl_2 (1/1) as the eluent to give compound 15k (2.47g, yield 67%).

^1H NMR (CDCl_3 , 400 MHz) δ 1.32-1.45 (m, 6H), 1.45-1.6 (m, 1H), 1.75-1.88 (2, 2H, rotamers), 2.01-2.11 (m, 2H), 2.82-2.87 (m, 1H), 3.33 (dd, $J = 13.4$ & 3.2 Hz, 1H), 4.10-4.28 (m, 2H), 4.62-4.72 (m, 1H), 4.90-5.05 (m, 3H), 5.73-5.88 (m, 1H), 7.17-7.38 (m,

5H).

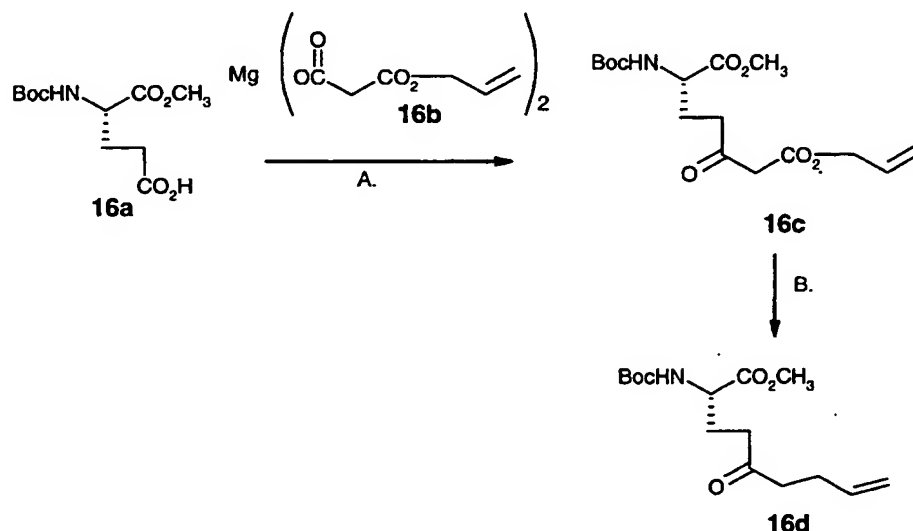
D. To a stirred solution of anhydrous SnCl_2 (2.61 g, 13.8 mmol) in anhydrous MeOH (80 mL), a solution of the azide **15k** (2.45 g, 6.9 mmol) was cannulated at 0° in anhydrous MeOH (20 mL). The mixture was stirred at RT for 4 h. The MeOH was
5 evaporated and the foamy material obtained was taken into dioxane/ H_2O (100 μL /20 μL) and treated with Boc_2O (3.0 g, 13.8 mmol) and NaHCO_3 (2.89 g, 34.5 mmol) (pH adjusted to 8 with more NaHCO_3 if needed) and the mixture was stirred at RT for 16 h. Part of the dioxane was evaporated (~50%) and the residue was extracted twice with EtOAc. The organic solution was washed with brine (2 x 50
10 mL), dried and evaporated. The residue obtained was chromatographed on silica gel using 20-25% EtOAc in hexane as eluent to give the compound **15l** (1.75 g, yield 60%).

^1H NMR (CDCl_3 , 400 MHz) δ 1.27-1.53 (m, 6H), 1.46 (s, 9H), 1.80 (m, 1H), 2.00-2.08 (m, 1H), 2.80 (t, $J = 12.1$ Hz, 1H), 3.34 (d, 14.3 Hz, 1H), 4.17-4.23 (m, 2H), 4.60-4.66
15 (m, 1H), 4.93 (d, $J = 10.2$ Hz, 1H), 5.05 (dd, $J = 17.2$ & 1.9 Hz, 1H), 5.13 (bs, 1H), 5.38-5.43 (m, 1H), 5.74-5.84 (m, 1H), 7.22-7.36 (m, 5H).

E. To a stirred solution at 90° of the N-Boc derivative **15l** (1.74 g, 4.04 mmol) in THF/ H_2O (75 mL/15 mL), H_2O_2 (30% v/w, 2.05 mL, 16.2 mmol) and $\text{LiOH}\cdot\text{H}_2\text{O}$ (0.34 g, 8.1 mmol) were added and the solution was stirred at 0° for 1 h. The
20 reaction was quenched with Na_2SO_3 (2.24 g in H_2O , 15 mL, 17.8 mmol). The pH was adjusted to 4-5 with 10% aqueous citric acid and the mixture diluted with EtOAc. The aqueous fraction was extracted once more with EtOAc and the organic solution was washed twice with brine, dried and evaporated. The residue was chromatographed on silica gel using 20% hexane in EtOAc as the eluent to give the
25 free carboxylic acid **15g** (0.76 g, yield 70%). This compound was identical in all respects to the one obtained in example 15.

EXAMPLE 16

Synthesis of (2S)-N-Boc-amino-5-oxo-non-8-enoic acid methyl ester (16d):



This synthesis is based on methodology by T. Tsuda et al., *J. Am. Chem. Soc.*, **1980**,
 5 102, 6381-6384.

A. To a well stirred solution of the monoallyl ester of malonic acid (1.50 g, 10.4 mmol) in dry THF under N_2 (20 mL) at -78° $n\text{-Bu}_2\text{Mg}$ (0.9M/hexane, 5.8 mL, 5.2 mmol) was added dropwise over a period of 5 min. The heavy suspension was then stirred at RT for 1 h and evaporated to dryness (vacuum release under N_2). The
 10 solid Mg salt 16b, was dried under vacuum for 1 h.

Glutamic acid derivative 16a was first mixed with 1,1'-carbonyldiimidazole (1.65 g, 10.21 mmol) in anhydrous THF and the mixture was stirred at RT for 1 h in order to activate the free acid moiety. Subsequently, the activated glutamic acid derivative was cannulated into a solution of the Mg salt 16b and the reaction mixture obtained
 15 was stirred at RT for 16 h. It was then diluted with EtOAc and the organic solution was washed with 0.5 N ice-cold HCl, brined, dried and evaporated. The residue obtained was chromatographed on silica gel using 35-40% EtOAc in hexane as eluent to give compound 16c (1.85 g, yield 53%).

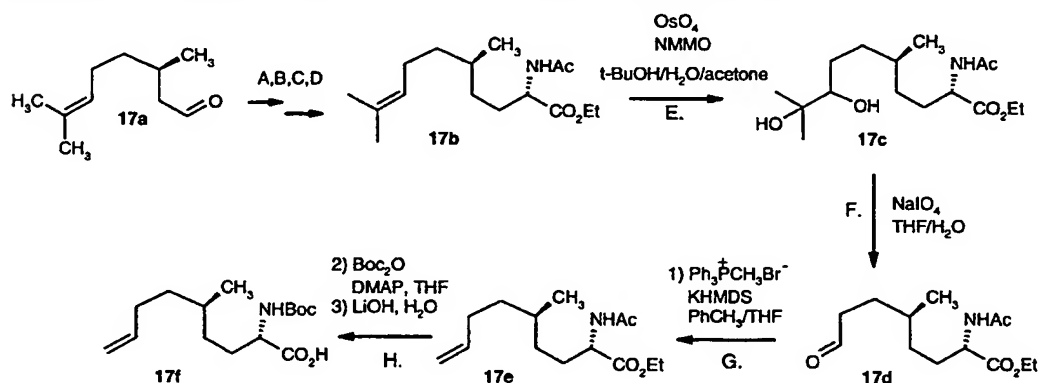
^1H NMR (CDCl_3 , 400 MHz) δ 1.44 (s, 9H), 1.85-1.95 (m, 1H), 2.12-2.22 (m, 1H), 2.58-
 20 2.74 (m, 2H), 3.48 (s, 2H), 3.74 (s, 3H), 4.24-4.34 (m, 1H), 4.52 (dm, $J = 5.7$ Hz, 2H), 5.09 (m, 1H), 5.25 (dm, $J = 10.2$ Hz, 1H), 5.34 (dm, $J = 17.2$ Hz, 1H), 5.91 (m, 1H).

B. To a stirred solution of tetrakis (triphenylphosphine) Pd (0) (0.116 g, 5 mol %, 0.1 mmole) in dry DMF (7 mL) was added (via a siring and under a N₂ atmosphere) the diester 16c (0.687 g, 2 mmol) in dry DMF (3 mL). The mixture was stirred at RT for 3.5 h. The DMF was evaporated under reduced pressure and the residue diluted with EtOAc (20 mL). The EtOAc solution was washed with 0.5 N ice-cold HCl (5 mL), brine (10 mL), dried and evaporated. The residue was chromatographed on silica gel using 15-20% EtOAc in hexane as eluent to give compound 16d (0.253 g, yield 42%).

¹H NMR (CDCl₃, 400 MHz) δ 1.44 (s, 9H), 1.84-1.94 (m, 1H), 2.08-2.22 (m, 1H), 2.33 (dd, J = 14.0 & 7.3 Hz, 2H), 2.45-2.55 (m, 4H), 3.74 (s, 3H), 4.28 (bm, 1H), 4.98 (dm, J = 10.2 Hz, 1H), 5.03 (dm, J = 17.2 Hz, 1H), 5.00-5.10 (m, 1H), 5.74-5.85 (m, 1H).

EXAMPLE 17

Synthesis of (2S,5R)-N-Boc-2-amino-5-methyl-non-8-enoic acid (17f)



A,B,C,D. Commercially available (R)-(+)-citronellal 17a was first converted to the amino acid derivative 17b following the same synthetic steps as those previously described in Example 15 for the conversion of aldehyde 15d to amino acid intermediate 15f.

E. Compound 17b (0.675 g, 5.6 mmol) was dissolved in a mixture of tBuOH/acetone/H₂O (1:1:1, 18 mL) and placed in an ice bath (0°C). NMMO (0.789 g, 6.74 mmol, 1.2 eq.) and OsO₄ (2.5% w/w in tBuOH, 0.7 mL, 0.067 mmol., 0.012 eq) were added consecutively and the reaction mixture was stirred at RT for 4 h. Most of the acetone was removed by evaporation under vacuum and then the mixture

was extracted with EtOAc. The organic layer was further washed with H₂O and brine, dried over anhydrous MgSO₄ and evaporated to dryness. The diol 17c was obtained in high purity after flash column chromatography using 1% EtOH in EtOAc as the eluent in 77% yield (0.575 g).

- 5 F. To a solution of diol 17c (0.575 g, 1.73 mmol) in THF/H₂O (1:1, 20 mL) at 0°C, NaIO₄ (0.48 g, 2.25 mmol, 1.3 eq.) was added and the reaction mixture was stirred at RT for 3.5 h. Most of the THF solvent was subsequently removed by evaporation under vacuum and the remaining mixture was extracted with EtOAc (2x100mL). The combined organic layers were further washed with 5% aqueous citric acid
10 solution (2x20 mL), 5% aqueous NaHCO₃ (20 mL) and brine (2x50 mL), then the EtOAc solution was dried over anhydrous MgSO₄ and evaporated to dryness under vacuum. The aldehyde intermediate 17d (0.47 g of crude product) was used in the next step without further purification.

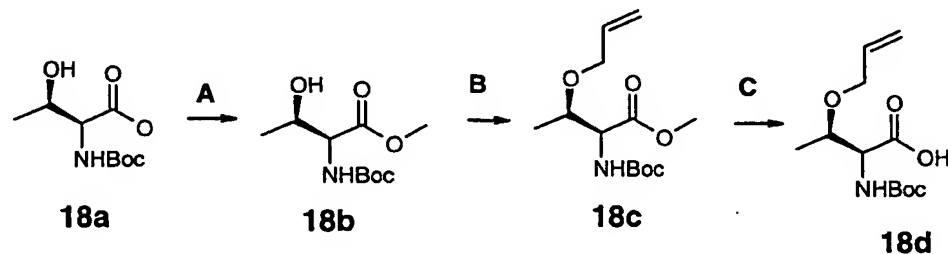
- G. To a solution of Ph₃PCH₃Br (925 mg, 2.6 mmol) in anhydrous toluene (15
15 mL), KHMDS (0.5M in toluene, 5.2 mL, 2.6 mmol) was added and the yellow suspension formed was stirred at RT for 30 min under N₂. After that period, the suspension was first cooled to 0°C, a solution of the aldehyde 17d (0.47 g 1.73 mmol, dissolved in 15 mL of anhydrous THF) was added *via* a syringe and the mixture was allowed to warm-up to RT. After stirring at RT for 1 h, most of the THF was
20 removed by evaporation under vacuum, EtOAc (100 mL) was added to the mixture and the organic layer was washed with H₂O (30 mL), 5% aqueous NaHCO₃ (30 mL) and brine (30 mL). The EtOAc solution was then dried over anhydrous MgSO₄ and evaporated to dryness under vacuum. Pure compound 17e was isolated after purification by flash column chromatography on silica gel, using hexane:EtOAc (3:2)
25 as the eluent, in 63% yield (0.29 g) for the two last steps.

The hydrolysis of the ethyl ester and simultaneous exchange of the *N*-acetyl protecting group for a Boc in intermediate 17e to obtain compound 17f was carried out using the same procedure as that reported for the conversion of compound 15f to 15g (17f, 310 mg, quantitative). ¹H NMR (CDCl₃, 400 MHz): δ 0.88 (d, J=6.4 Hz,

3H), 1.18-1.28 (m, 2H), 1.35-1.48 (m, 3H), 1.45 (s, 9H), 1.64-1.74 (m, 1H), 1.81-1.89 (m, 1H), 1.94-2.12 (m, 2H), 4.28 (bd, $J \approx 3.2$ Hz, 1H), 4.93 (dm, $J = 11.1$ Hz, 1H), 5.00 (dm, $J = 16.8$ Hz, 1H), 5.74-5.84 (m, 1H).

EXAMPLE 18

5 Synthesis of *N*-Boc-*O*-allyl-(*L*)-threonine (18d)



- A. Boc-(*L*)-threonine **18a** (500 mg, 2.28 mmol) was partially dissolved in $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (8 mL/0.5 mL, respectively) at 0°C . A solution of diazomethane in diethyl ether was slowly added until the yellow color persisted, indicating the presence of excess diazomethane. Upon evaporation of the solvents, crude methyl ester **18b** was obtained as a cloudy white oil (0.534 g).
- B. Intermediate **18b** (311 mg, 1.33 mmol) was then dissolved in anhydrous diethyl ether (8 mL), Ag_2O was added (341 mg, 1.47 mmol) and freshly activated 4Å molecular sieves (1 g). Finally, allyl iodide (134 μL , 1.47 mmol) was added to the reaction flask and the mixture was stirred at reflux. Two more portions of allyl iodide (45 μL , 0.50 mmol, each time) were added after a period of 20 h and 30 h, and stirring was continued for a total of 36 hours. Then the mixture was filtered through celite and purified by flash column chromatography on silica gel, using EtOAc/hexane (1:4) as the eluent, to give 73 mg (27% yield) of compound **18c** as a clear oil.

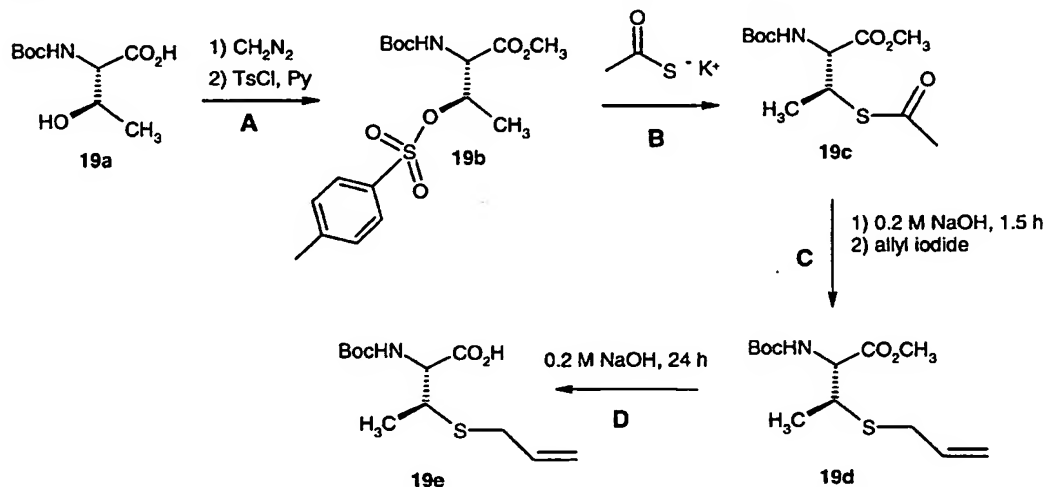
^1H NMR (CDCl_3 , 400MHz): δ 1.21 (d, $J = 6.0$ Hz, 3H), 1.45 (s, 9H), 3.75 (s, 3H), 3.82-3.87 (m, 1H), 3.99-4.07 (m, 2H), 4.29 (dd, $J = 9.5$ & 2.5 Hz, 1H), 5.14 (dm, $J = 10.5$ Hz, 1H), 5.21 (dm, $J = 17.2$ Hz, 1H), 5.75-5.84 (m, 1H).

- C. The ester compound **18c** (99 mg, 0.362 mmol) was dissolved in a mixture of THF/MeOH/ H_2O (2:1:1, 4 mL) and $\text{LiOH}\cdot\text{H}_2\text{O}$ (61 mg, 1.45 mmol) was added. The solution was stirred at RT for 2 h, and was then acidified with 1N HCl to pH ~ 3

before the solvents were removed under vacuum. The resulting oil, compound 18d was used as such for the synthesis of macrocyclic inhibitors.

EXAMPLE 19

Synthesis of (2S, 3S)-N-Boc-2-amino-3(mercaptoallyl)butanoic acid (19e)



5

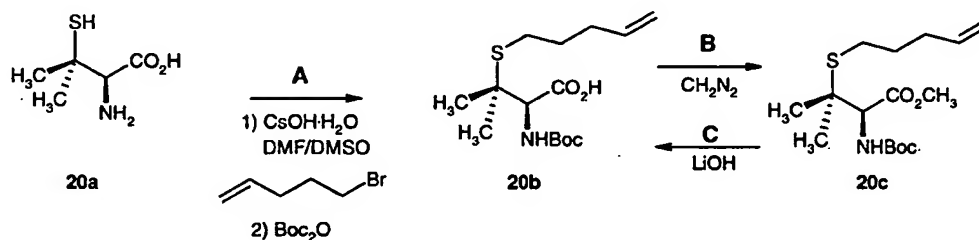
- A. Compound 19a (9.1 mmol) was dissolved in pyridine (5 mL) and the solution was cooled to 0°C in an ice bath, tosyl chloride (2.3 g, 11.8 mmol, 1.3 eq.) was added in small portions and the reaction mixture was stirred at RT for 24 h. After that period, the reaction mixture was partitioned between diethyl ether (300 mL) and H₂O (100 mL). The ether layer was further washed with 0.2N HCl (6x100 mL) and brine (100 mL), dried over anhydrous MgSO₄, filtered and concentrated to dryness under vacuum. Purification of the crude material by flash column chromatography, using hexane/EtOAc (gradient from 8:2 to 7:3 ratio) as the eluent, led to the isolation of tosyl derivative 19b in 85% yield (3.05 g).
- 15 B. To solution of intermediate 19b (775 mg, 2 mmol) in anhydrous DMF (2.5 mL), potassium thioacetate (365 mg, 3.2 mmol, 1.6 eq.) was added and the reaction mixture was stirred at RT for 24 h. Most of the DMF was then evaporated under vacuum and the remaining mixture was partitioned between EtOAc and H₂O. The aqueous layer was re-extracted with EtOAc, the combined organic layers were
- 20 washed with brine, dried over anhydrous MgSO₄ and evaporated to dryness. Purification of the crude material by flash column chromatography using hexane/EtOAc (4:1 ratio) as the eluent, led to the isolation of compound 19c in 80%

yield (465 mg).

- C. To a solution of thioester **19c** (465 mg) in H₂O/EtOH (3:5 ratio, 8 mL), an aqueous solution of 0.2M NaOH (2.4 mL) was added and the mixture was stirred at RT for 1.5 h. Allyl iodide (0.292 mL, 3.2 mmol, 2 eq.) was then added and stirring
- 5 was continued at RT for an additional 30 min. The reaction mixture was concentrated to half of its original volume and then extracted with EtOAc. The aqueous layer was acidified to pH=~3 with cold aqueous 0.5N HCl and re-extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous MgSO₄ and evaporated to dryness under vacuum. The crude reaction
- 10 mixture contained at least four products; all of the products were isolated after flash column chromatography on silica gel, using hexane/EtOAc (gradient from 9:1 to 3:1 ratio). The structure of the least polar compound (TLC R_f = 0.68 in hex/EtOAc 4:1) corresponded to the desired product **19d** (83 mg, 18% yield).
- ¹H NMR (CDCl₃, 400 MHz): δ 1.24 (d, J=7.0 Hz, 3H), 1.46 (s, 9H), 3.13-3.19 (m, 2H),
- 15 3.24-3.29 (m, 1H), 3.77 (s, 3H), 4.50 (dd, J=8.6 & 3.8 Hz, 1H), 5.12 (d, J=12.4 Hz, 1H), 5.15 (dd, J=18.4 & 1.3 Hz, 1H), 5.22 (bd, J=7.6 Hz, 1H), 5.75-5.85 (m, 1H).
- D. A solution of the methyl ester **19d** (83 mg, 0.287 mmol) in MeOH/H₂O (3:1, 4 mL) was mixed with aqueous NaOH (0.2 N, 1.3 mL, 0.26 mmol) for 24 h at RT and for 1 h at 40 °C. The reaction mixture was acidified with cold aqueous HCl (0.5 N
- 20 HCl, at 0 °C, pH=4-5), the MeOH was removed under vacuum and the remaining aqueous mixture was extracted with EtOAc. The organic solution was dried over MgSO₄ and evaporated to dryness in order to obtain compound **19e**. Compound **19e** was used for the final synthesis of inhibitors without any further purification.

EXAMPLE 20

Synthesis of (S)-N-Boc-2-amino-3-methyl-3-(1-mercapto-4-butenyl)butanoic acid (20c)



5 A. L-Penicillamine 20a (448 mg, 3 mmol) was dissolved in DMF/DMSO (5:1 ratio, 6 mL), 4-bromopentene (0.46 mL, 4.5 mmol, 1.5 eq.) and CsOH•H₂O (1.0g, 6mL, 2 eq.) were added and the reaction mixture was stirred at RT. After 24 h, Boc₂O (820 mg, 3.75 mmol, 1.25 eq.) was added to the mixture and stirring was continued for an additional 12 h. The DMF was subsequently removed under vacuum, the remaining
 10 mixture was diluted with cold aqueous 0.5N HCl adjusting the pH=4-5 and then extracted with EtOAc (2x50 mL). The organic layer was washed with brine (2x), dried over anhydrous MgSO₄ and evaporated to dryness to give the crude carboxylic acid 20b.

B. Purification of 20b turned out to be difficult, thus the crude product was first
 15 treated with diazomethane to form the corresponding methyl ester 20c, and then purified by flash column chromatography, using hexane/EtOAc (9:1) as the eluent, to obtain 190 mg (20% yield) of the pure methyl ester 20c.

¹H NMR (400 MHz, CDCl₃): δ 1.35 (s, 3H), 1.37 (s, 3H), 1.44 (s, 9H), 1.59-1.67 (m, 2H),
 2.11-2.17 (m, 2H), 2.51-2.60 (m, 2H), 3.74 (s, 3H), 4.29 (d, J= 8.6 Hz, 1H), 4.98 (dm,
 20 J=10.5 Hz, 1H), 5.03 (dm, J=19 Hz, 1H), 5.35 (bd, J=7 Hz, 1H), 5.72-5.83 (m, 1H).

C. The ester was subsequently dissolved in THF/MeOH/H₂O (2:2:1, 5mL), LiOH•H₂O (50 mg, 2.0 mmol, 2 eq.) was added and the reaction mixture stirred at
 40°C for 4 h to hydrolyze the ester 20c back to the acid 20b. The reaction mixture was acidified with 0.5N HCl to pH=4-5, the THF and MeOH were evaporated to
 25 dryness and the remaining aqueous solution was extracted with EtOAc. The EtOAc

layer was dried over anhydrous MgSO_4 and evaporated to dryness to give compound **20b**, which was used in the subsequent synthesis of macrocyclic inhibitors without further purification.

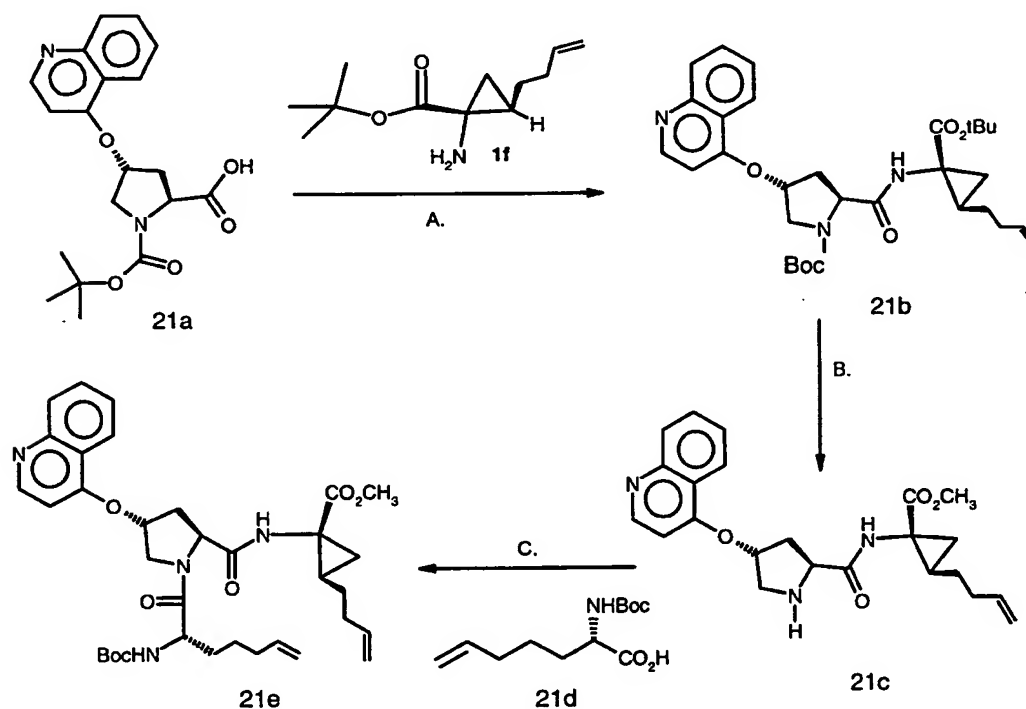
5 ACYCLIC DIPEPTIDE AND TRIPEPTIDE INTERMEDIATES

The general procedure for coupling reactions done in solution and specific examples thereof are described in WO 00/09543 and WO 00/09558.

These procedures have been used for the synthesis of the intermediate dipeptides **26c**, **30a** and tripeptides **23a**, **24a**, **31a**, **32a**, and **33a**.

10 EXAMPLE 21

Synthesis of acyclic tripeptide **21e**



- A. To a solution of the proline derivative **21a** (prepared from commercially available Boc-4(R)-hydroxyproline and 4-chloro-quinoline as described in WO 00/05543 and WO 00/09558) (1.32 g, 3.68 mmol) and the crude homoallyl ACCA **1f** (~3.35 mmol) in CH_2Cl_2 (10 mL), NMM (1.21 mL, 10.05 mmol) and HATU (1.53, 4.02 mmol) were added in succession and the suspension was stirred at RT for 18 h. After that period, the solvent was evaporated and the crude reaction mixture was

redissolved in EtOAc (30 mL). The solution was washed with 5% aqueous NaHCO₃ (2 x 10 mL), brine (10 mL), dried over MgSO₄ and evaporated. The crude product was purified by chromatography on silica gel using 8% diethyl ether in EtOAc as the eluent to obtain the desired diastereomer of compound 21b in 20% yield (the absolute stereochemistry was not determined).

¹H NMR (CDCl₃, 400 MHz): δ 0.93 & 1.01 (t, J = 8.3 Hz, 1H, rotamers in ratio of 3:7), 1.14-1.35 (m, 2H), 1.44 (s, 9H), 1.45 (s, 9H), 1.50-1.82 (m, 4H), 2.08-2.24 (m, 2H), 2.32 (bs, 0.7H), 2.63 (bs, 0.75H), 2.93 (bs, 0.75H), 3.16 (m, 0.25H), 3.77 (bs, 1.5H), 3.88 (bs, 0.5H), 4.4-4.55 (m, 1H), 4.98 (d, J = 10.2 Hz, 1H), 5.03 (dd, J = 17.2 & 1.6 Hz, 1H), 5.24 (bs, 1H), 5.75-5.88 (m, 1H), 6.57 & 6.78 (2bs, 1H, 2 rotamers), 7.42-7.58 (m, 3H), 7.63-7.73 (m, 2H), 8.04 (d, J = 8.3 Hz, 1H), 8.11 (d, J = 8.3 Hz, 1H), 8.74 (d, J = 5.1 Hz, 1H).

B. To a solution of the dipeptide 21b (137 mg, 0.248 mmol) in dry CH₂Cl₂, a solution of HCl in dioxane (4M, 4 mL) was added and the mixture was stirred at RT for 1.5 h. The solvent was then evaporated and the residue dried under high vacuum to give the free amino acid. The mixture was dissolved in diethyl ether/MeOH (3 µL/2 µL) and treated with a slight excess of diazomethane dissolved in diethyl ether. After 30 min, the excess diazomethane was destroyed with the addition of HCl (4M in dioxane) and the mixture was evaporated to dryness to obtain the HCl salt of compound 21c which was used in the next step without any purification.

C. To a stirred suspension of the crude dipeptide 21c (0.23 g, 0.48 mmole) in CH₂Cl₂ (25 mL) was added in succession the (2S)-N-Boc-amino-hept-6-enoic acid 21d (0.151 g, 0.62 mmol), NMM (210 µL, 1.91 mmol) and HATU (0.236 g, 0.62 mmole) and the mixture was stirred at RT for 16 h (the pH was adjusted to ~8 with NNM after 1 h if needed). The CH₂Cl₂ was evaporated, the residue taken into EtOAc (50 mL) and the organic solution washed with 5% NaHCO₃ (2 x 20mL), brine (2 x 20mL), dried and evaporated. The crude compound obtained was chromatographed on silica gel (50 mL, 2% EtOH/EtOAc) to give compound 21e (0.139 g, yield 46%).

¹H NMR (CDCl₃, 400 MHz, rotamers in 6:1 ratio) chemical shifts of major rotamer δ

1.21-1.27 (m, 1H), 1.36 (s, 9H), 1.45-1.81 (4m, 7H), 2.20-2.22 (m, 4H), 2.28-2.37 (m, 1H), 2.90-2.99 (m, 1H), 3.66 (s, 3H), 3.94-3.98 (m, 1H), 4.29 (bd, J = 9.9 Hz, 1H), 4.46-4.50 (m, 1H), 4.81 (dd, J = 8.3 & 5.4 Hz, 1H), 4.92-5.06 (m, 4H), 5.16 (d, J = 8.3 Hz 1H), 5.37 (m, 1H), 5.70-5.84 (m, 2H), 6.82 (d, J = 5.1 Hz, 1H), 7.47-7.55 (m, 2H), 7.71 (dt, J = 7.0 & 1.3 Hz, 1H), 8.03 (d, J = 8.6 Hz, 1H), 8.17 (d, J = 8.0 Hz, 1H), 8.78 (d, J = 5.1 Hz, 1H).

MACROCYCLIC PEPTIDES

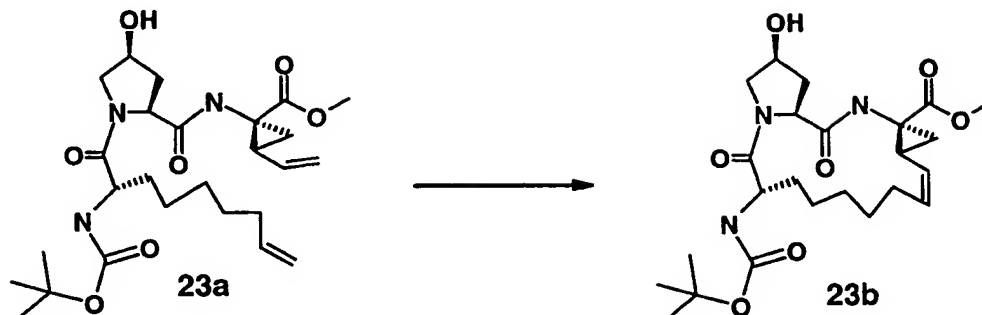
EXAMPLE 22

General procedure for macrocyclization via olefin metathesis

- 10 In all cases, the tri-peptide diene was dissolved in CH_2Cl_2 at a concentration of 0.01M and the solution was deoxygenated by the bubbling of argon (~1 h for a volume of 500 mL). A solution of catalyst (5-30 mol %, dissolved in a small amount of degassed CH_2Cl_2) is added and the reaction mixture is refluxed until all starting material was converted to product(s) as indicated by TLC and HPLC. The crude
- 15 reaction mixtures were subsequently concentrated to near dryness and filtered through a short pad of silica gel, eluting first with CH_2Cl_2 to remove most of the catalyst and then with EtOAc in order to elute all of the macrocyclic product(s) (most of the time as a single diastereomer). The crude product(s) from each reaction is analyzed by chiral HPLC on a CHIRALCEL OJ-R column (purchased from Chiral
- 20 Technologies Inc, 0.46 ϕ x 15 cm), using an isocratic solvent mixture of 70% H_2O + 0.06% TFA – 30% CH_3CN + 0.06% TFA at 205 nm. The major macrocyclic product(s) was fully characterized by: ^1H , COSY, TOCSY, and ROESY NMR data in order to confirm its structure and stereochemistry.

EXAMPLE 23

Synthesis of macrocyclic intermediate (23b)



A solution of diene 23a (4.0 g, 7.88 mmol) in dry CH_2Cl_2 (800 mL, Aldrich-anhydrous) was deoxygenated by bubbling Ar for 2 h. Hoveyda's catalyst (262 mg, 0.434 mmol, 5.5 mol %) was then added as a solid and the reaction was refluxed under an Ar balloon. After 28 h, the red-orange solution was evaporated to an amorphous solid and then purified by flash column chromatography over silica gel.

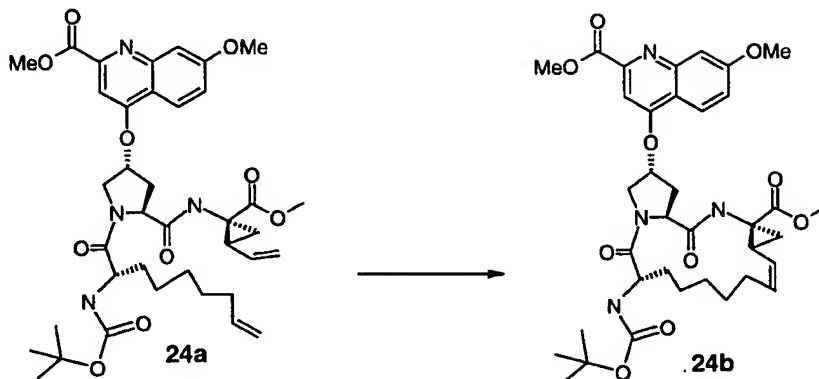
The initial solvent system was 10% EtOAc in CH_2Cl_2 . Once the catalyst was eluted from the column, the solvent was changed to pure EtOAc. Elution of the catalyst from the column was evident from its color. The macrocyclic product 23b was isolated as a colorless foam which was re-dissolved in CH_2Cl_2 /hexane (~1:2).

Evaporation of the solvent afforded a white powder (3.362 g, 89% yield).

^1H NMR (CDCl_3 , 400 MHz): δ 1.20-1.50 (m, 6H), 1.43 (s, 9H), 1.53 (dd, $J = 9.5$ & 5.4, 1H), 1.61-1.70 (m, 1H), 1.76-1.90 (m, 2H), 2.05-2.26 (m, 4H), 2.45 (d, $J = 14.3$, 1H), 3.67 (s, 3H), 3.71 (d, $J = 11.1$, 1H), 3.90 (dd, $J = 11.1$ & 4.3, 1H), 4.43-4.53 (m, 2H), 4.76 (d, $J = 8.6$, 1H), 4.86 (bd, $J = 9.8$, 1H), 5.20-5.23 (m, 2H), 5.57 (dt, $J = 7.0$ & 9.8, 1H), 7.32 (bs, 1H).

EXAMPLE 24

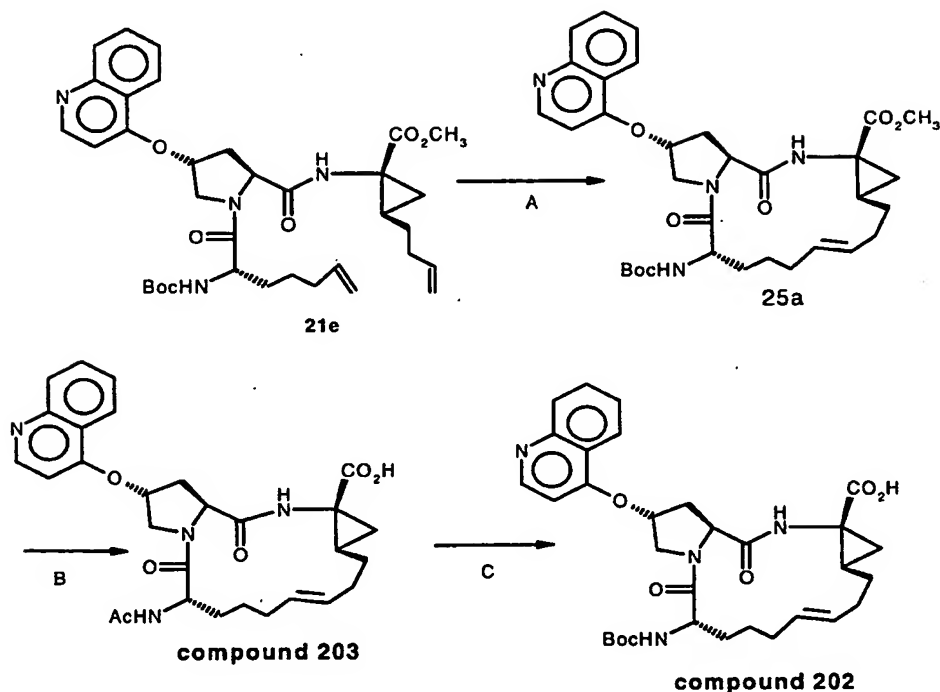
Synthesis of macrocyclic intermediate (24b):



- A solution of diene **24a** (2.76 g, 3.82 mmol) in anhydrous CH_2Cl_2 (600 mL, anhydrous) was deoxygenated by bubbling Ar for 1.5 h. A solution of Hoveyda's catalyst (117 mg, 0.19 mmol, 0.05 eq) in anhydrous and degassed CH_2Cl_2 (8 mL) was added *via* cannula and the reaction was stirred at reflux under an Ar balloon. After 20 h, the reaction mixture was approximately 50% completed, at which point a second portion of catalyst was added (117 mg) and the stirring was continued for an additional 16 h. The solution was then concentrated to ~ 100 mL, applied to the top of a pad of silica gel (6 x 10 cm) and the catalyst was first recovered by eluting with CH_2Cl_2 . Compound **24b** was washed off the pad of silica with 3% MeOH in EtOAc and re-purified by flash column chromatography using EtOAc/hexane (2:1) to obtain 70% yield of a slightly olive-tinted white solid (1.85 g, 94% pure by HPLC).
- ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.69 (s, 1H), 8.13 (d, $J = 9.2$ Hz, 1H), 7.50-7.44 (m, 2H), 7.17 (dd, $J = 9.2, 2.2$ Hz, 1H), 7.04 (d, $J = 6.4$ Hz, 1H), 5.60-5.56 (m, 1H), 5.52 (dd, $J = 9.2$ Hz, 1H), 5.25 (dd, $J = 9.2$ Hz, 1H), 4.59 (d, $J = 11$ Hz, 1H), 4.44 (dd, $J = 9.2$ Hz, 1H), 4.05-3.98 (m, 1H), 3.94 (s, 3H), 3.92 (s, 3H), 3.89-3.82 (m, 1H), 3.55 (s, 3H), 2.64-2.53 (m, 1H), 2.46 (d, $J = 7.3$ Hz, 1H), 2.40-2.31 (m, 1H), 2.21 (dd, $J = 8.9$ Hz, 1H), 1.78-1.65 (m, 2H), 1.55 (dd, $J = 4.8$ Hz, 1H), 1.485 (dd, $J = 4.8$ Hz, 1H), 1.41-1.30 (m, 7H), 1.16 (s, 9H). MS; es^+ : 795.4 ($\text{M} + \text{H}$) $^+$.

EXAMPLE 25

Synthesis of compound 202 & 203 (Table 2)



- A. The diene compound 21e (0.130 g, 0.205 mmol) was cyclized using catalytic amounts of bis-(tricyclohexylphosphine) benzylidene ruthenium IV dichloride (Grubb's catalyst, *supra*) (52 mg, 0.064 mmol) in CH_2Cl_2 (60 mL) under reflux for 2 h to give after chromatography on silica gel (50 mL, 3% EtOH/EtOAc) compound 25a (60.1 mg, yield 48%).

^1H NMR (CDCl_3 , 400 MHz) δ 1.22-1.30 (m, 2H), 1.35 (s, 9H), 1.44-2.35 (m, 13H), 3.07-3.14 & 3.16-3.24 (2m, 1H, rotamers in 1:3 ratio), 3.69 (s, 3H), 3.96-4.04 (m, 1H), 4.42-4.50 (m, 1H), 4.95-5.04 (m, 1H), 5.05-5.15 (m, 1H), 5.20-5.30 (m, 1H), 5.55-5.65 (m, 1H), 6.75-6.79 (2d, $J = 5.4$ Hz, 1H, rotamers in 1:3 ratio), 7.36 (s, 1H), 7.46-7.50 (m, 1H), 8.03 (d, $J = 8.3$ Hz, 1H), 8.13 & 8.17 (2d, $J = 8.0$ Hz, 1H, rotamers in 1:3 ratio), 8.77 (d, $J = 5.1$ Hz, 1H).

- B. The ester moiety of the macrocyclic compound 25a (0.0156 g, 0.026 mmol) was hydrolyzed with $\text{LiOH} \cdot \text{H}_2\text{O}$ (8.7 mg, 0.206 mmol) in THF/MeOH/ H_2O (4 mL / 2 mL / 2 mL). The crude product was purified by C18 reversed phase HPLC on a Whatman (Partisil 10,0DS3) 50/2.4 cm column using a solvent gradient from 5%

72

aqueous CH₃CN to 100% CH₃CN to obtain pure compound 202 as an amorphous white solid (11.8 mg).

¹H NMR (DMSO, 400 MHz): δ 1.12 (s, 9H), 1.20-1.24 (m, 2H), 1.32-1.40 (m, 3H), 1.58-1.62 (m, 2H), 1.68-1.78 (m, 3H), 1.95-2.02 (m, 1H), 2.08-2.18 (m, 2H), 2.42-2.59 (m, 2H), 3.97-4.00 (bd, J = 9.8 Hz, 2H), 4.47 (t, J = 8.6 Hz, 1H), 4.58 (d, J = 11.8 Hz, 1H), 5.22-5.29 (m, 1H), 5.46-5.54 (m, 1H), 5.66 (s, 1H), 7.12 (d, J = 6.0 Hz, 1H), 7.49 (d, J = 3.5 Hz, 1H), 7.68 (dd, J = 7.3 Hz, 1H), 7.98 (dd, J = 7.0 Hz, 1H), 8.08 (d, J = 8.3 Hz, 1H), 8.21 (s, 1H), 8.35 (d, J = 8.3 Hz, 1H), 9.08 (d, J = 5 Hz, 1H).

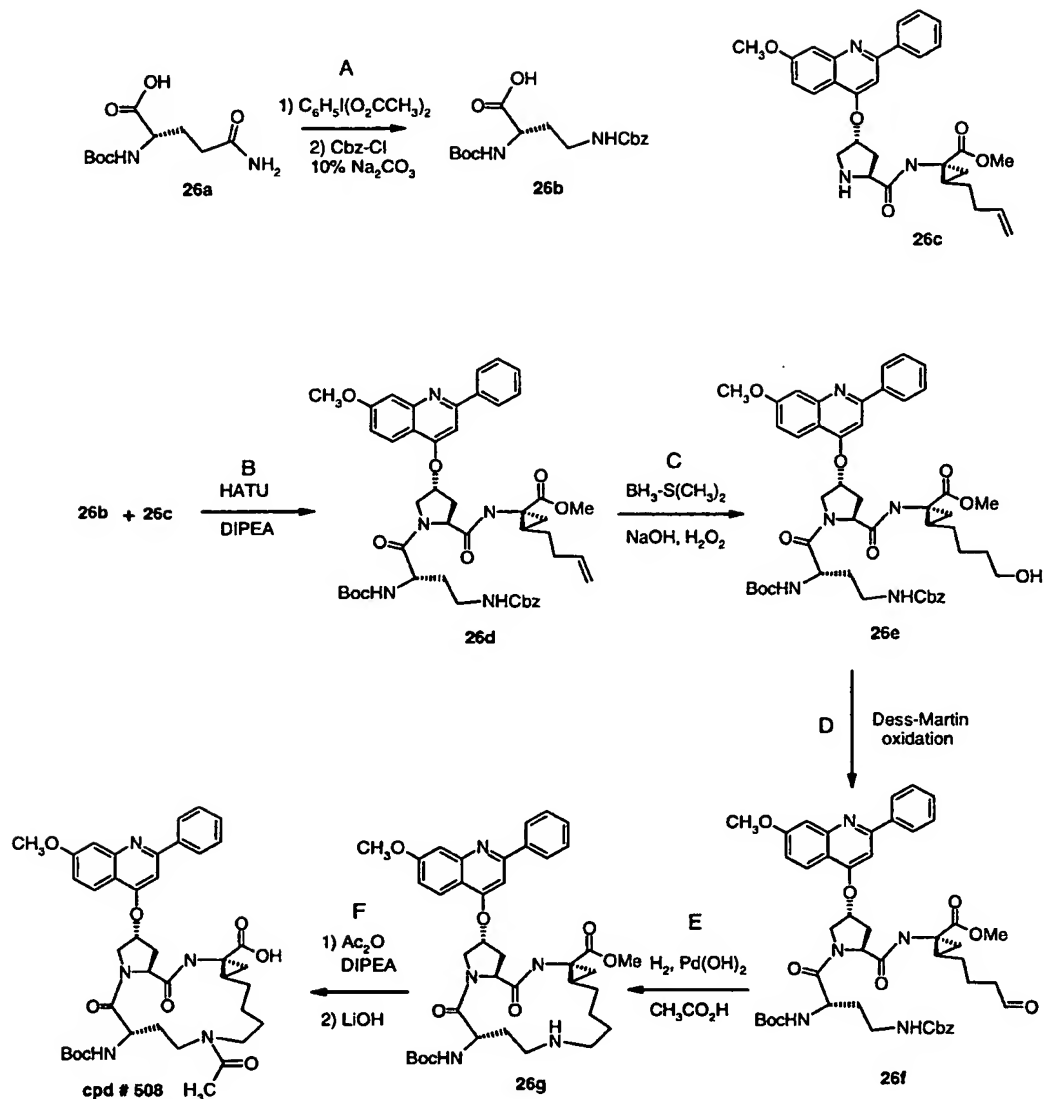
C. The macrocyclic compound 25a (20 mg, 0.033 mmol) in dry CH₂Cl₂ (1 mL) was stirred in presence of 4M HCl/dioxane (5 mL) for 1 h. The mixture was evaporated and dried carefully. The residue was re-dissolved in CH₂Cl₂/DMF (3 mL / 1 mL) and treated with NMM (14.5 μL, 0.132 mmol) and acetic anhydride (7.0 μL, 0.073 mmol) and stirred at RT for 14 h. The mixture was evaporated and dried under high vacuum. The residue was then dissolved in a mixture of THF/MeOH/H₂O (4 mL / 2 mL / 2 mL) and stirred overnight with LiOH.2H₂O (11 mg, 0.264 mmol). The residue isolated after acidification to pH = 3 with 1N ice-cold HCl was purified by C18 reversed phase HPLC using a solvent gradient from 0-40% aqueous CH₃CN (0.06% TFA) in order to isolated pure compound 203 as an amorphous white solid (12 mg).

¹H NMR (50mM Na₂PO₄ buffer, pH=6.0, 600 MHz): δ 1.22-1.27 (m, 2H), 1.38-1.43 (m, 2H), 1.58-1.64 (m, 2H), 1.67-1.76 (m, 2H), 1.77-1.84 (m, 1H), 1.92-1.99 (m, 1H), 2.22-2.08 (m, 1H), 2.12-2.27 (m, 1H), 2.22-2.27 (m, 1H), 2.60-2.67 (m, 1H, Pro-β'), 2.83-2.89 (m, 1H, Pro-β), 4.32 (dd, J = 12.1 & 3.5 Hz, 1H, Pro-δ'), 4.41 (dd, J = 12.1 & 7.3 Hz, 1H), 4.56 (bd, J = 8.0 Hz, 1H, Pro-δ), 4.62 (dd, J = 8.9 Hz, 1H, Pro-α), 5.40-5.46 (m, 1H), 5.55-5.61 (m, 1H), 5.73 (bs, 1H, Pro-γ), 7.41 (d, J = 6.3 Hz, 1H), 7.64 (bs, 1H, Acca-NH), 7.80 (dd, J = 7.9 Hz, 1H), 8.03 (dd, J = 8.0 Hz, 1H), 8.07 (d, J = 9.5 Hz, 1H), 8.16 (d, J = 7 Hz, 1H, AcNH), 8.36 (d, J = 8.3 Hz, 1H), 8.90 (d, J = 6.0 Hz, 1H).

73

EXAMPLE 26

Synthesis of compound 508 (Table 5)



- A. A solution of Boc-protected L-glutamine 26a (4.93 g, 20 mmol) and
 5 iodobenzene diacetate (7.73 g, 24 mmol, 1.2 eq.) in EtOAc/ CH_3CN / H_2O (2:2:1, 60 mL), was stirred at 16°C for 1 h and at 20°C for 3 h. The reaction mixture was then diluted with H_2O (20 mL), the EtOAc and CH_3CN solvents were removed under vacuum and the remaining aqueous mixture was extracted with diethyl ether (3x50 mL) and EtOAc (50 mL) in order to remove most of the impurities. The aqueous
 10 layer (containing the amine intermediate) was then concentrated to dryness, the

- remaining material was re-dissolved in 10% Na₂CO₃ (30 mL), cooled to 0°C in an ice bath and a solution of benzyl chloroformate (3.3 mL, 20.4 mmol, 1.02 eq.) dioxane (40 mL) was slowly added (~10 min). The reaction mixture was stirred at 0°C for 1 h and at RT for 2 h. The mixture was then diluted with H₂O (50 mL), extracted with
- 5 cold (~5°C) diethyl ether (3x50 mL), acidified with 4M HCl to pH=3-4 and extracted with EtOAc (3x50 mL). The combined organic layers were dried over anhydrous MgSO₄ and evaporated to dryness under vacuum. The crude material was purified by flash column chromatography, using EtOAc/Hexane/AcOH (7:2.9:0.1) to obtain compound 26b in 43% overall yield (3.04 g).
- 10 B. Dipeptide intermediate 26c (250 mg, 0.41 mmol), compound 26b (171 mg, 0.49 mmol, 1.2 eq.) and HATU (185 mg, 0.49 mmol, 1.2 eq.) were dissolved in CH₂Cl₂ (6 mL) and DIPEA (0.29 mL, 1.62 mmol, 4 eq.) was added. The reaction mixture was stirred at RT for 14 h, then the CH₂Cl₂ was evaporated under vacuum and the crude material re-dissolved in EtOAc. The EtOAc solution was washed with aqueous 5%
- 15 NaHCO₃ and brine, dried over anhydrous MgSO₄ and evaporated to dryness. Compound 26d was obtained after purification of the crude material by flash column chromatography, using EtOAc/hexane (4:1) as the eluent, in 98% yield (338 mg).
- C. A solution of compound 26d (335 mg, 0.394 mmol) in THF (5 mL) was cooled
- 20 to 0°C and a solution of BH₃ in dimethyl sulfide (0.12 mL of 10M solution, 1.2 mmol, 3 eq.) was added. The reaction mixture was allowed to warm-up to RT and stir for 1 h. Then it was cooled again to 0°C before an aqueous solution of NaOH (0.8 mL of 2.5 M solution, 1.97 mmol, 5 eq) was added slowly over a period of 15 min, followed by the slow addition (~15 min) of an aqueous solution of H₂O₂ (0.8 mL of an 8.8 M
- 25 solution, 6.9 mmol, 17.5 eq.). The reaction mixture was allowed to warm-up to RT and stir for 1 h. After that period, the reaction mixture was acidified to pH ~4 in order to quench the excess BH₃, then aqueous NaHCO₃ was added to adjust the pH=~9-10, the THF was removed under vacuum and the crude material was partitioned between H₂O and EtOAc. The aqueous layer was re-extracted with

75

EtOAc, the combined organic layers were washed with brine, dried over anhydrous MgSO_4 and evaporated to dryness under vacuum. The crude material was purified by flash column chromatography, using EtOAc/hexane/ NH_4OH (8:2:0.5) as the eluent, to obtain pure compound 26e in 57% yield (192 mg).

- 5 D. To a solution of compound 26e in CH_2Cl_2 (8 mL), Dess-Martin periodinate (195 mg, 97%, 0.33 mmol, 1.5 eq) was added and the reaction mixture was stirred at RT for 1.5 h. The reaction was quenched with the addition of aqueous $\text{Na}_2\text{S}_2\text{O}_3$ (3 mL of 5% solution), then saturated aqueous NaHCO_3 (5 mL) was added and the mixture was stirred at RT for 15 min. Finally, the reaction crude was extracted with
- 10 EtOAc, the organic layer was washed with aqueous 5% NaHCO_3 and brine, dried over anhydrous MgSO_4 and evaporated under vacuum to give 188 mg of aldehyde 28f which was used in the next step without further purification.

- E. A solution of compound 26f (188 mg, 0.22 mmol), $\text{CH}_3\text{CO}_2\text{H}$ (38 μL) and $\text{Pd}(\text{OH})_2$ (25 mg) in ethanol (5 mL) was stirred to RT under H_2 at atmospheric
- 15 pressure for 16 h. After that period, more H_2 gas, $\text{Pd}(\text{OH})_2$ (180 mg) and $\text{CH}_3\text{CO}_2\text{H}$ (154 μL) and were added to the flask and stirring was continued for an additional 24 h. The mixture was then filtered and the solvent evaporated to dryness, the crude macrocyclic product was purified by flash column chromatography, using $\text{CHCl}_3/\text{MeOH}/\text{AcOH}$ (10:2:1), to obtain compound 26g in ~30% yield (48 mg).

- 20 F. A mixture of compound 26g (22 mg, 0.031 mmol), DIPEA (27 μL , 0.155 mmol, 5 eq.) and acetic anhydride (8.7 μL , 0.093 mmol, 3 eq.) in CH_2Cl_2 (5 mL) was stirred at RT for 16 h. The CH_2Cl_2 was then removed under vacuum, a mixture of THF/MeOH/ H_2O (2:2:1, 5 mL) and $\text{LiOH}\cdot 2\text{H}_2\text{O}$ (13 mg, 0.31 mmol, 10 eq.) were added and the hydrolysis reaction was allowed to proceed for 68 h at RT and 2 h at
- 25 50°C . The reaction mixture was then acidified ($\text{pH} \sim 4$) and purified by reversed phase HPLC to obtain the final compound 508 (~6 mg, ~26% yield for the last 2 steps).

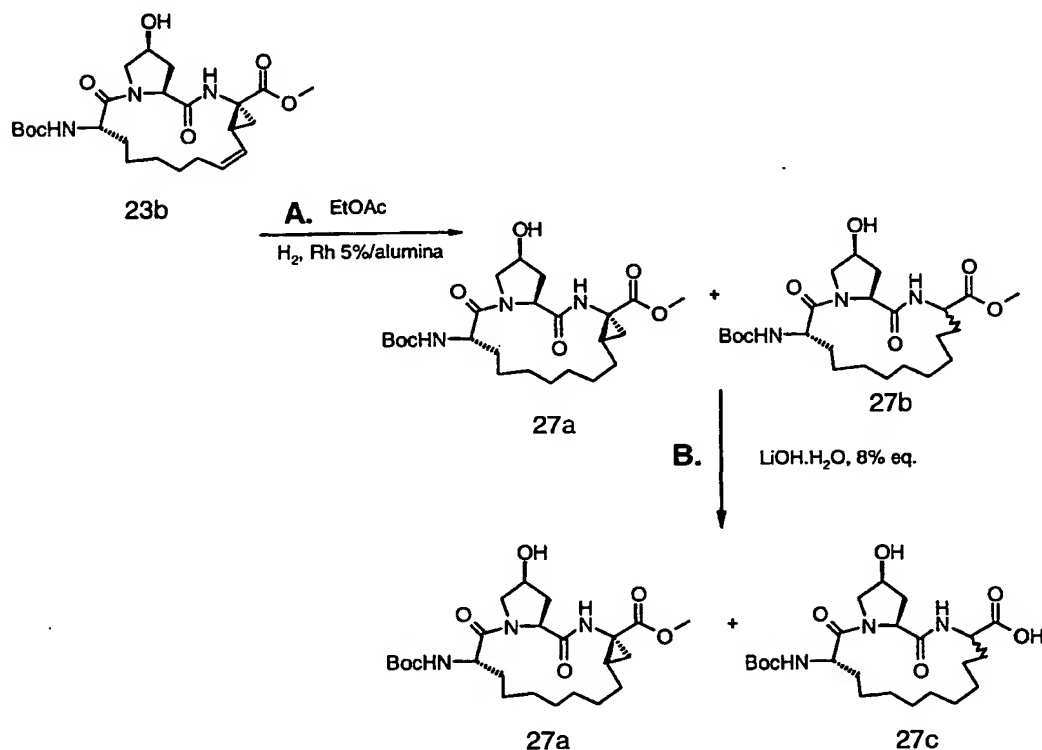
^1H NMR (DMSO, 400 MHz) of 508 (mixture of rotamers confirmed by COSY,

76

TOCSY and ROESY NMR data): δ 1.18 (s, 9H), 1.09-1.85 (overlapping m, 11H), 1.95 (s, 3H), 2.30 (m, 1H), 2.63 (m, 1H), 3.18-4.14 (overlapping m, 6H), 3.96 (s, 3H), 4.44 (m, 1H), 4.62 & 4.69 (2d, $J = 11.8$ Hz, 1H, rotamers), 5.82 (bs, 1H), 7.20 (m, 2H), 7.53 (bs, 1H), 7.67 (bs, 4H), 8.19 (bs, 3H), 8.61 (s, 1H).

5 EXAMPLE 27

Synthesis of the saturated macrocyclic intermediate (27a)



- A. The unsaturated macrocyclic intermediate **23b** (3.50 g, 7.30 mmol) was dissolved in EtOAc (30 mL) and 700 mg (20% w/w) of 5% Rh on alumina was added. The mixture was stirred under H₂ gas at atmospheric pressure and at RT for 1.5 h. After that period, HPLC analysis confirmed the complete conversion of starting material to two products, the desired product **27a** and a minor product (8% of the total mass) which was later identified to be compound **27b**, formed from opening of the cyclopropane ring. The reaction mixture was filtered and concentrated to give a light green color solid (3.47 g). The solid was co-evaporated twice with EtOH to remove all of the EtOAc (the presence of EtOAc interferes in the next step). Separation of compound **27a** from **27b** by chromatography proved to be

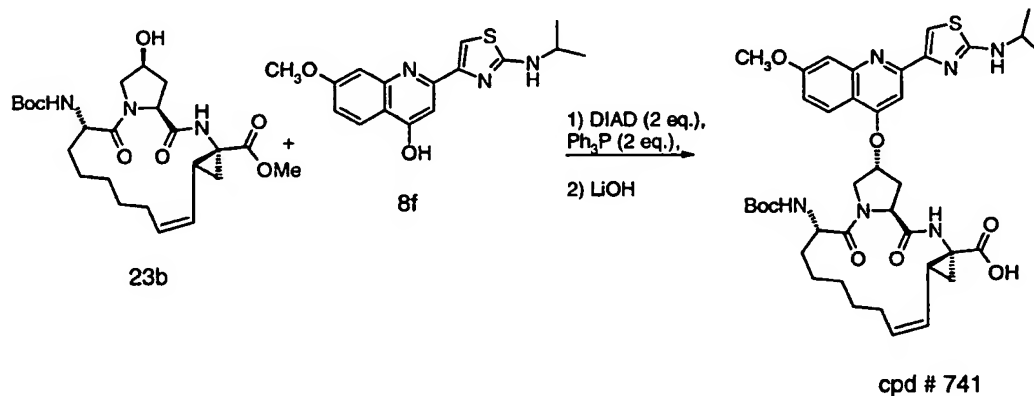
very difficult, thus an alternative method was devised based on the relative rates of hydrolysis of their respective methyl ester moieties.

- B. The crude mixture of compounds 27a and 27b (3.47g) was dissolved in THF:MeOH (1:1, 20 mL), an aqueous solution of LiOH•H₂O (24 mg in 5 mL H₂O, 8% eq) was added and the reaction mixture was stirred at RT for 16 h (complete hydrolysis of the side product 27b to its corresponding acid 27c was confirmed by HPLC). The reaction mixture was concentrated under vacuum in order to remove most of the THF and MeOH and partitioned between H₂O (100 mL) and EtOAc (300 mL). The organic layer was washed with 0.5 N NaOH (3x100 mL), brine (100 mL), 10% aqueous citric acid (2x100 mL), brine (100 mL), dried over anhydrous MgSO₄, filtered and concentrated to dryness. The desired product 27a was obtained in high purity (>90% by HPLC) as a light green foam and in 93% overall yield (3.28 g) for the two steps.

- ¹H NMR: (400 MHz, CDCl₃): δ 1.1-1.38 (m, 13 H), 1.42 (s, 9 H), 1.51-1.57 (m, 1 H), 1.63-1.67 (dd, J = 8.0 & 5.1 Hz, 1 H), 1.81-1.87 (m, 1 H), 1.92-1.99 (m, 1 H), 2.02-2.08 (m, 1 H), 2.62 (d, J = 14 Hz, 1 H), 3.4 (d, J = 8.3, 1H), 3.65 (s, 3H), 4.01 (dd, J = 10.8 & 4.1 Hz, 1 H), 4.42-4.48 (m, 1 H), 4.51-4.55 (m, 1 H), 4.87 (d, J = 8.6 Hz, 1 H), 5.14 (d, J = 8.6 Hz, 1 H), 7.97 (br s, 1 H).

EXAMPLE 28

- 20 Synthesis of compound #741 (Table 7)



Quinoline derivative 8f was attached to the pre-formed macrocyclic compound 23b via a Mitsunobu reaction. The quinoline derivative 8f (30 mg, 0.095 mmol) was

78

dissolved in THF, then macrocycle 23b (45.6 mg, 1 eq.) and PPh_3 (49.8 mg, 2 eq.) are then added. The resulting mixture is cooled to 0°C . DIAD (37.4 μl , 2 eq.) is then added dropwise. The solution is stirred 1 hour at 0°C then stirred overnight at room temperature. The mixture was then diluted with EtOAc (15 ml), washed with a saturated solution of NaHCO_3 (15 ml), followed by brine. The solution was dried with MgSO_4 , filtered and concentrated in vacuo. 202 mg of a yellow oil was obtained. The product was purified by flash chromatography on silica gel (100% EtOAc). The product still contained DIAD byproducts after the purification. The resulting product obtained contained 55% w/w of the desired product, so the yield was declared to be 62%.

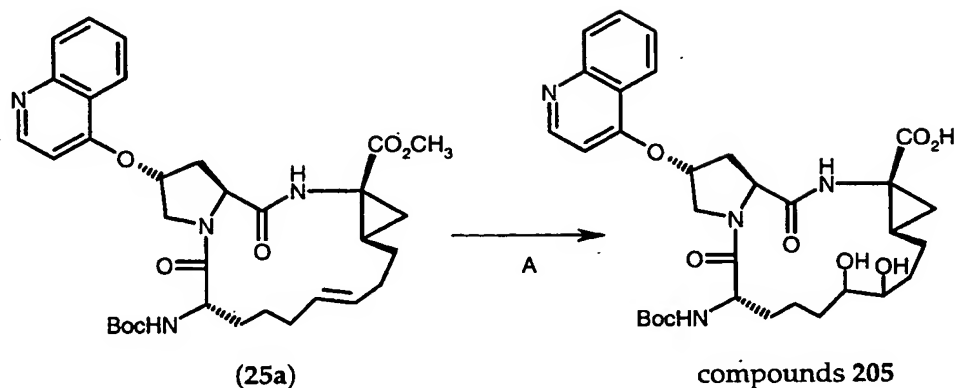
The ester intermediate (46 mg, 0.06 mmol) was dissolved in a mixture of THF/MeOH/ H_2O (2:1:1 ratio, 2 mL), $\text{LiOH}\cdot\text{H}_2\text{O}$ (20 mg, 0.48 mmol) was added and the solution was stirred at RT. After a period of 16 h, analysis of the reaction mixture by HPLC indicated that the hydrolysis was complete. The organic solvents were removed under vacuum and the remaining crude material dissolved in DMSO was purified by C18 reversed phase HPLC to give pure inhibitor 741.

^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ (ppm): 8.67 (s, 1H), 8.29-8.14 (m, 2H), 8.08-7.97 (m, 1H), 7.91-7.78 (m, 1H), 7.74 (s, 1H), 7.31-7.20 (m, 1H), 7.10 (d, $J = 5.7$ Hz, 1H), 5.82-5.71 (m, 1H), 5.58-5.47 (m, 1H), 5.32-5.23 (m, 1H), 4.74-4.64 (m, 1H), 4.55-4.47 (m, 1H), 4.23-4.06 (m, 1H), 4.04-3.94 (m, 1H), 3.97 (s, 3H), 3.92-3.85 (m, 1H), 2.70-2.55 (m, 2H), 2.53-2.36 (m, 2H), 2.20-2.09 (m, 1H), 1.80-1.62 (m, 2H), 1.56-1.43 (m, 2H), 1.42-1.29 (m, 6H), 1.27 (d, $J = 3.2$ Hz, 3H), 1.25 (d, $J = 2.9$ Hz, 3H), 1.12 (s, 9H).

MS: 763.1 (M+1), 761.1 (M-1).

EXAMPLE 29

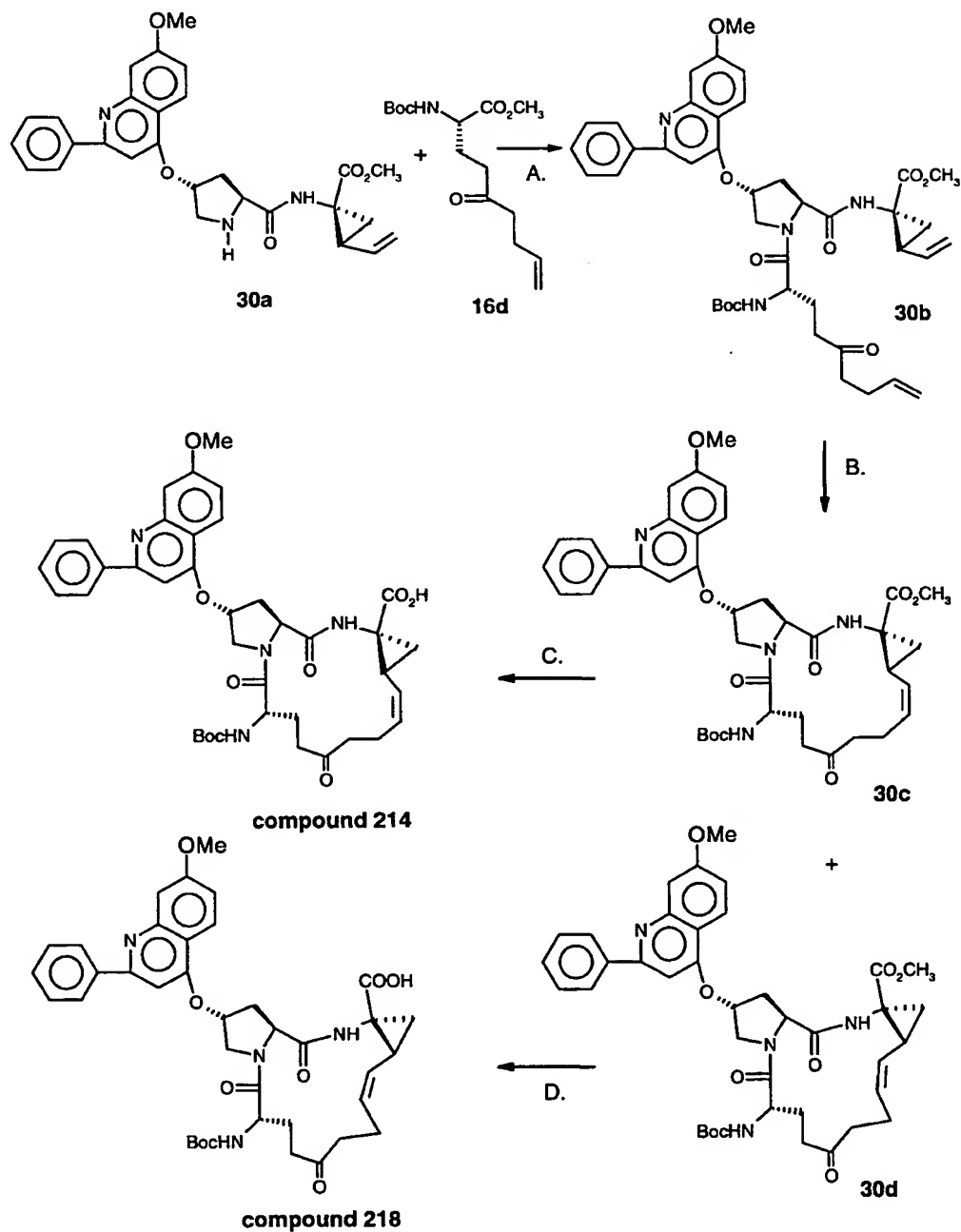
Synthesis of compound 205 (Table 2)



- 5 To a solution of the macrocyclic compound 25a (21 mg, 0.035 mmol) in t-butanol/ H_2O (1.5 mL/1.5 mL) at 0° , a solution of OsO_4 in t-butanol (0.36 mL of a 35% w/v, 0.035 mmol) was added and the mixture was stirred at RT for 1 h. The mixture was diluted with EtOAc (20 mL) and the organic solution washed with 5% NaHCO_3 (2 x 10 mL), brine (2 x 10 mL), dried and evaporated to dryness. The crude
- 10 compound was taken into THF/MeOH/ H_2O (3 mL / 1.5 mL / 1.5 mL) and stirred in presence of $\text{LiOH}\cdot\text{H}_2\text{O}$ (13 mg, 0.28 mmol) for 16 h. The mixture was acidified to pH 4 with 0.5 N ice-cold HCl, evaporated and purified by C18 reversed phase HPLC using a solvent gradient from H_2O (0.06% TFA) to 40% aqueous CH_3CN (0.06% TFA). The syn diol 205 was isolated in high purity as amorphous white solid.
- 15 compound #205: ^1H NMR (DMSO, 400 MHz): δ 1.01 (s, 9H), 1.06-1.30 (m, 9H), 1.48-1.68 (m, 3H), 1.78-1.88 (m, 1H), \approx 2.2-2.5 (2m, 2H), 3.78-3.82 (m, 1H), 3.86-3.90 (m, 1H), 4.39 (t, J = 8.9 Hz, 1H), 4.61 (d, J = 11.4 Hz, 1H), 5.60 (bs, 1H, Pro- γ), 7.03 (d, J = 6.0 Hz, 1H), 7.40 (bs, 1H), 7.58-7.62 (m, 1H), 7.87-7.91 (m, 1H), 8.00 (d, J = 8.3 Hz, 1H), 8.24 (d, J = 8.6 Hz, 1H), 8.60 (s, 1H), 8.99 (bs, 1H).
- 20 EMS (negative ionization mode): m/z 625 (M-H) $^-$.

EXAMPLE 30

Synthesis of compound 214 & 218 (Table 2)



- 5 A. A solution of the keto-nonenoate ester 16d (0.180 g, 0.6 mmol) in MeOH/H₂O (5 mL / 2 mL) was stirred at RT in presence of LiOH•H₂O (50 mg, 1.2 mmol) for 1 h. The solution was acidified to pH 6 with 0.5 N ice-cold HCl and most of the MeOH was evaporated. The residue was then dissolved in EtOAc (30 mL)

and the solution was washed with 0.5 N ice-cold HCl (10 mL), brined (10 mL), dried and evaporated. The crude residue was then re-dissolved in CH₂Cl₂ (10 mL) and reacted with the P1-P2 fragment 30a (0.337 g, 0.6 mmol) in the presence of HATU (233 mg, 0.612 mmol) and DIPEA (420 µL, 2.4 mmol) over a period of 16 h at RT.

- 5 The reaction mixture was chromatographed on silica gel using EtOAc/hexane (1/1) as the eluent, to isolate the pure compound 30b (0.370 g, yield 83%, Purity >95% by HPLC).

¹H NMR (CDCl₃, 400 MHz) δ 1.41 (s, 9H), 1.45-1.54 (m, 1H), 1.58-1.62 (m, 1H), 1.73-1.77 (m, 1H), 1.86-1.91 (m, 1H), 2.16 (dd, J = 17.8 & 8.6 Hz, 1H), 2.26-2.43 (2m, 2H),
10 2.46-2.58 (m, 2H), 2.64-2.81 (m, 1H), 2.85-2.92 & 2.95-3.03 (2m, 1H, rotamers in 1:3 ratio), 3.67 (s, 3H), 3.95 (s, 3H), 4.10-4.18 (m, 1H), 4.20-4.30 (m, 1H), 4.40-4.55 (m, 1H), 4.80-4.88 (m, 1H), 4.92-5.10 (m, 2H), 5.14 (dd, J = 10.2 & 1.6 Hz, 1H), 5.24-5.38 (m, 4H), 5.42-5.54 (m, 1H), 5.68-5.86 (m, 2H), 7.04-7.14 (m, 2H), 7.42-7.64 (m, 5H), 7.92-8.12 (m, 3H).

- 15 B. The diene 30b (0.370 g, 0.49 mmol) was cyclized in the presence of the bis-(tricyclohexylphosphine) benzylidene ruthenium IV dichloride catalyst (0.125 mg, 0.15 mmol) in CH₂Cl₂ (distilled from CaH₂ and degassed with argon for 30 min) over a period of 2 h at reflux. The compound was obtained as a mixture of stereoisomers (30c and 30d 1:1 ratio) after flash column chromatography on silica gel using
20 EtOAc/Hexane (3/1) in 35% yield (0.124 g).

¹H NMR of mixture 30c & 30d (CDCl₃, 400 MHz) δ 1.44 (s, 4H) & 1.37 (s, 4H), 1.60 (m, 2H), 1.83 (m, 0.5H), 2.01 (m, 1H), 2.09 (m, 1H), 2.42 (m, 5H), 2.73 (m, 2H), 3.26 (m, 0.5H), 3.69 (s, 1.5H), 3.76 (s, 1.5H), 3.96 (s, 3H), 4.10 (m, 1H), 4.24 (m, 0.5H), 4.10 (m, 0.5H), 4.58 (m, 1H), 4.73 (m, 1H), 4.89 (m, 0.5H), 4.97 (m, 0.5H), 5.30 (m, 0.5H),
25 5.44 (m, 2H), 5.64 (m, 1H), 7.1-7.0 (m, 3H), 7.47 (m, 4H), 8.08-7.98 (m, 3H).

- C,D. Hydrolysis of the methyl esters 30c and 30d (24 mg, 0.033 mmol) was carried out in THF/MeOH/H₂O (1 mL / 0.5 mL / 0.5 mL) with LiO•H₂O (11 mg, 0.246 mmol) over a period of 16 h at RT. After that period the reaction mixture was acidified to pH 4-5 and chromatographed on a C18 reversed phase HPLC column
30 using a solvent gradient from H₂O (0.06% TFA) to 50% aqueous CH₃CN (0.06% TFA). The desired compounds 214 and 218 were isolated from the mixture of the

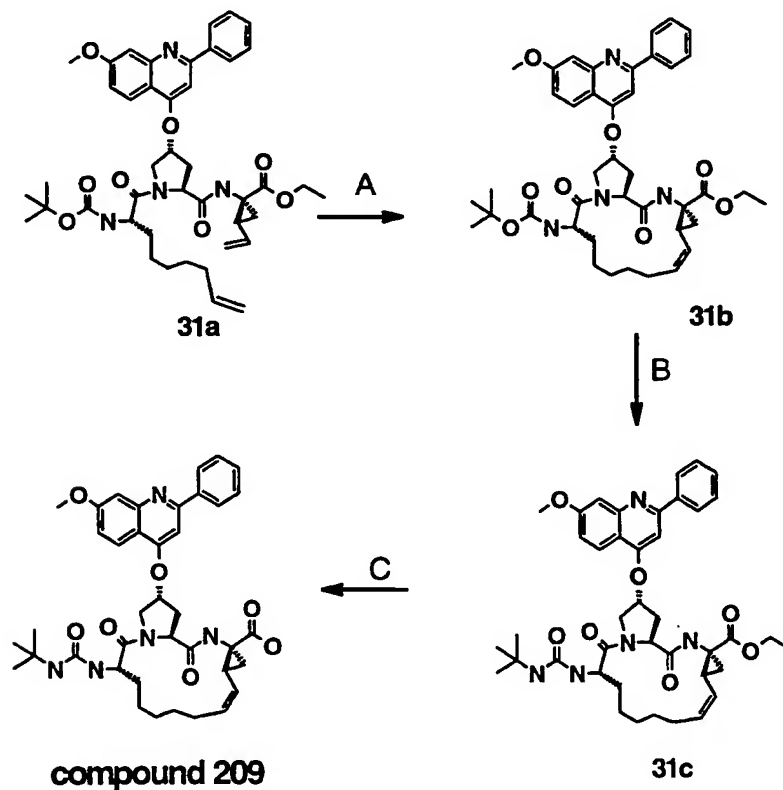
two compounds in high purity (94% pure by HPLC) in 15% yield (3 mg).

compound **214**: ^1H NMR (DMSO, 400 MHz) δ 1.15 (s, 9H), 1.48-1.54 (m, 2H), 1.65-1.74 (m, 1H), 1.77-1.85 (m, 1H), 2.12-2.25 (m, 4H), 2.27-2.34 (m, 1H), 2.61-2.68 (m, 1H), 2.87 (bt, $J = 11.5$ Hz, 1H), 3.92 (dd, $J = 9.2$ & 1.5 Hz, 1H, Pro- δ), 3.97 (s, 3H, $-\text{OCH}_3$), 4.14-4.20 (m, 1H), 4.52 (t, $J = 7.8$ Hz, 1H, Pro- α), 4.66 (d, $J = 11.8$ Hz, 1H, Pro- δ), 5.45 (t, $J = 9.9$ Hz, 1H), 5.51-5.58 (m, 1H), 5.82 (bs, 1H, Pro- γ), 7.09 (d, $J = 6.0$ Hz, 1H, BocNH), 7.26 (bs, 1H), 7.53 (s, 1H), 7.67 (bs, 3H), 8.16 (d, $J = 2$ Hz, 1H), 8.18 (s, 1H), 8.83 (s, 1H, ACCA-NH).

compound **218**: ^1H NMR(DMSO, 400 MHz): δ 1.06-1.10 (m, 1H), 1.18 (s, 9H), 1.52-1.55 (m, 1H), 1.62-1.80 (m, 1H), 2.10-2.68 (overlapping, 9H), 3.90 (bd, $J = 8.3$ Hz, 1H), 3.96 (s, 3H, OCH_3), 4.20-4.27 (m, 1H), 4.58-4.63 (m, 1H, Pro- δ), 4.66 (dd, $J = 8.3$ Hz, 1H, Pro- α) 4.88 (dd, $J = 10.2$ Hz, 1H), 5.18-5.26 (m, 1H), 5.73-5.79 (m, 1H, Pro- γ), 7.01 (d, $J = 6.4$ Hz, 1H), 7.23 (bs, 1H), 7.50 (bs, 1H), 7.66 (bs, 3H), 8.20 (bs, 2H), 8.53 (s, 1H).

EXAMPLE 31

15 Synthesis of compound 209 (Table 2)



83

A. The diene 31a (249 mg, 0.330 mmol) was dissolved in 30 mL of anhydrous CH_2Cl_2 and the solution was degassed with argon for 15 min. The catalyst bis-(tricyclohexylphosphine) benzylidene ruthenium IV dichloride (82 mg, 0.100 mmol) was dissolved in 3 mL of anhydrous and degassed CH_2Cl_2 and added to the diene

5 solution. The reaction mixture was refluxed for 2 h under N_2 . The solution was concentrated and purified by flash column chromatography to obtain compound 31b as a brown solid in 71% yield (171 mg).

^1H NMR (CDCl_3 , 400 MHz): δ 1.22-1.44 (m, 10H), 1.42 (s, 9H), 1.66-1.74 (m, 1H), 1.87-1.97 (m, 2H), 2.13-2.28 (m, 3H), 2.32-2.39 (m, 1H), 3.08-3.16 (m, 1H), 3.41 (s, 3H),
10 4.07-4.22 (m, 3H), 4.28-4.34 (m, 1H), 4.58-4.64 (m, 1H), 4.95-4.99 (m, 1H), 5.22-5.29 (m, 2H), 5.38-5.43 (m, 1H), 5.48-5.56 (m, 1H), 7.00-7.12 (m, 3H), 7.43-7.55 (m, 4H), 7.97-8.11 (m, 3H).

ES(+)-MS: m/z 727.4 ($\text{M}+\text{H}$) $^+$.

B. Compound 31b (0.117 mmol) was stirred in a solution of HCl (1 mL of 4N in dioxane) for 30 min and concentrated to dryness. The solid was taken up in CH_2Cl_2 (2 mL), Et_3N (82 μL , 0.585 mmol) and t-butylisocyanate (35 mg, 0.351 mmol) were successively added. After stirring at RT for 20 h, the mixture was concentrated to dryness and the crude compound 31c was used in the final hydrolysis step without further purification.

20 D. Compound 31c (85 mg, 0.117 mmol) was dissolved in THF/MeOH/ H_2O (2 mL / 1 mL / 1 mL), $\text{LiOH}\cdot\text{H}_2\text{O}$ (39 mg, 0.936 mmol) was added and the solution was stirred for 20 h at RT. After that period, acetic acid (1 mL) was added and the solution was concentrated to remove the MeOH and THF. The pure compound 209 was isolated after purification of the crude by C18 reverse phase HPLC (25 mg,
25 ~31% yield).

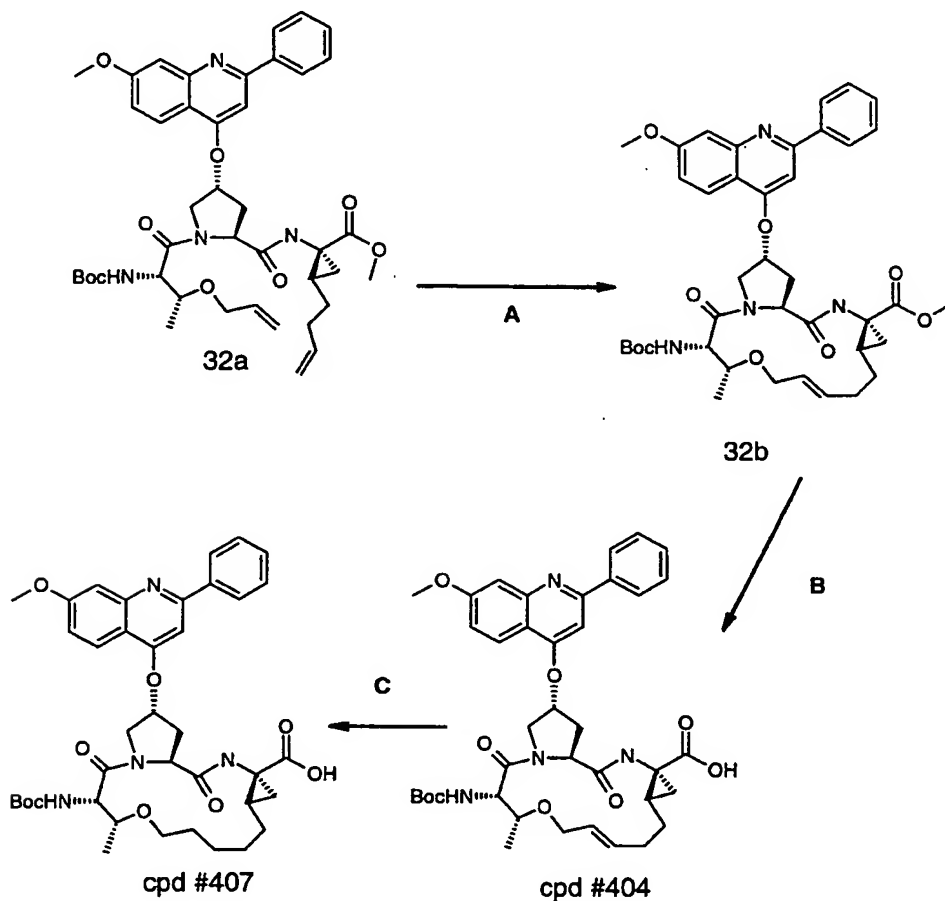
^1H NMR (DMSO, 400 MHz): δ 1.04 (s, 9H), 1.15-1.24 (m, 2H), 1.30-1.40 (m, 5H), 1.44-1.51 (m, 2H), 1.54-1.68 (m, 1H), 1.75-1.88 (m, 1H), 2.18 (dd, $J = 17.2$ & 8.5 Hz, 1H), 2.32-2.45 (m, 1H, Pro- β), 2.54-2.62 (m, 1H), 2.65-2.68 (m, 1H, Pro- β), 3.91 (dd, $J = 11.1$ & 3.5 Hz, 1H, Pro- δ), 3.96 (s, 3H, $-\text{OCH}_3$), 4.17-4.23 (m, 1H), 4.47 (dd, $J = 8.6$, 1H, Pro-

α), 4.67 (bd, $J = 7.9$ Hz, 1H, Pro- δ), 5.30 (dd, $J = 9.5$ Hz, 1H), 5.52 (bdd, $J = 19$ & 8.3, 1H), 5.68 (s, 1H), 5.78 (bs, 1H, Pro- γ), 5.94 (bs, 1H), 7.21 (bs, 1H), 7.51 (bs, 1H), 7.66 (bs, 4H), 8.19 (s, 2H), 8.40 (d, $J = 7$ Hz, 1H), 8.61 (s, 1H, ACCA-NH).

ES(+)-MS: m/z 698.3 ($M+H$)⁺.

5 EXAMPLE 32

Synthesis of compounds #404 and #407 (Table 4)



- A. The diene 32a (84 mg, 0.11 mmol) was dissolved in anhydrous CH_2Cl_2 (11 mL) and the solution was degassed over a period of 15 min. with a flow of argon.
10. The bis-(tricyclohexylphosphine) benzylidene ruthenium IV dichloride catalyst (19 mg, 0.023 mmol) was first dissolved in 1 mL of degassed CH_2Cl_2 and then it was transferred to the reaction flask *via* cannula. The reaction mixture was stirred for 2 h at reflux. The solvent was then removed under vacuum and the reaction mixture was purified by flash column chromatography on silica gel, using EtOAc/ hexane

(1:1) as the eluent, to give the macrocyclic compound **32b** as a yellow oil (33 mg, 41% yield).

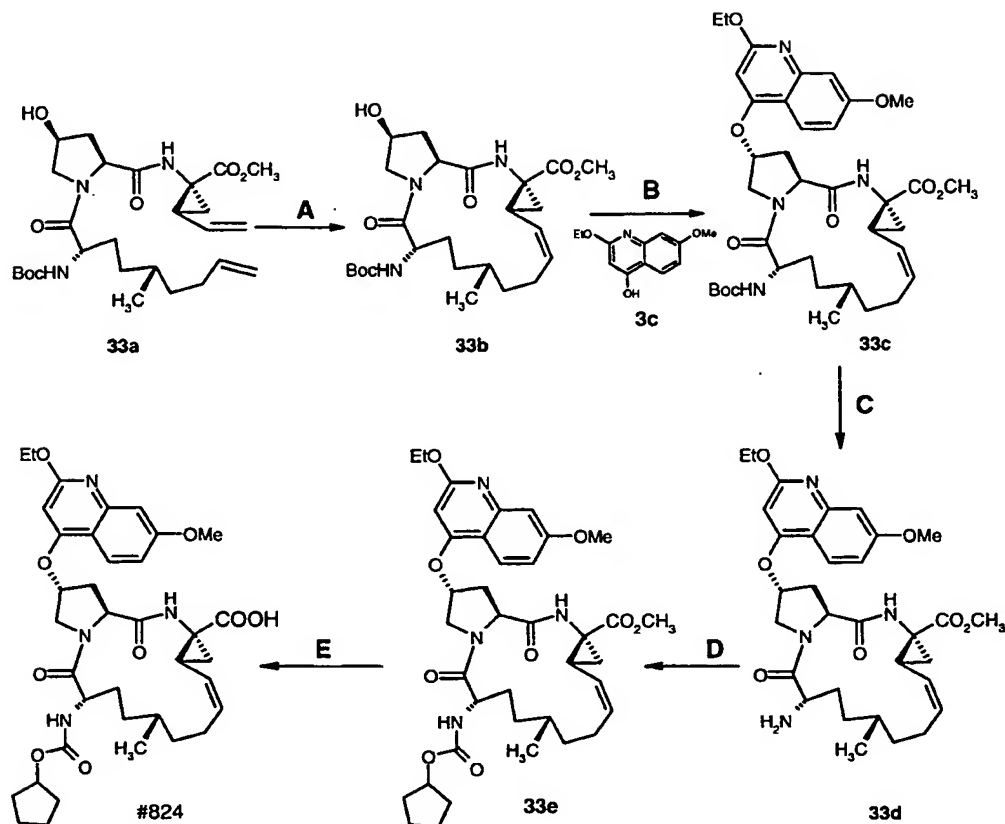
B. The ester intermediate **32b** (33 mg, 0.045 mmol) was dissolved in a mixture of THF/MeOH/H₂O (2:1:1 ratio, 2 mL), LiOH•H₂O (8 mg, 0.18 mmol) was added
5 and the solution was stirred at RT. After a period of 16 h, analysis of the reaction mixture by HPLC indicated that the hydrolysis was incomplete. Thus an additional amount of LiOH•H₂O (4 mg, 0.09 mmol) was added and the solution was stirred at RT for a total of 36 h. Finally, the solution was acidified with a small aliquot of acetic acid, the organic solvents were removed under vacuum and the remaining
10 crude material was purified by C18 reversed phase HPLC to give pure inhibitor **404**.
¹H NMR (DMSO, 400MHz): δ 1.21 (d, J = 6.0 Hz, 3H, Me), 1.36 (s, 9H, Boc), 1.1-1.4 (3m, 3H), 1.66 (m, 1H), 1.80 (m, 1H), 2.10 (m, 2H), 2.57 (m, 2H), 3.90 (m, 4H), 4.47 (bd, J=12.7 Hz, 1H), 4.58 (bd, J=7.3, 1H), 4.66 (dd, J=8.0 Hz, 1H), 5.57 (m, 1H), 5.66 (m, 1H), 5.83 (bs, 1H), 6.18 (bd, J=6.9 Hz, 1H), 7.25 (bd, J=7.3 Hz, 1H), 7.56 (bs, 1H),
15 7.70 (m, 4H), 8.22 (bd, J=2.9 Hz, 2H), 8.29 (bs, J=9.2 Hz, 1H).

C. Inhibitor **404** (15 mg, 0.021 mmol) was dissolved in ethanol (2 mL) and Pd 10%/C (2 mg) was added. The mixture was stirred under hydrogen at RT for 16 h. After filtration, the mixture was purified by C18 reversed phase HPLC to give inhibitor **407** as a white solid (10 mg, 66% yield)

20 ¹H NMR (DMSO, 400MHz): δ 1.04 (m, 1H), 1.17 (d, J = 6.0 Hz, 3 H), 1.35 (s, 9H), 1.25-1.75 (m, 12 H), 2.32-2.45 (m, 1 H), 3.40-3.50 (m, 2 H), 3.74-3.83 (m, 1H), 3.85-3.93 (m, 1H), 3.97 (s, 3H), 4.27-4.36 (dd, J = 21.1 & 8.6 Hz, 1H), 4.54 (dd, J = 7.95 & 7.95 Hz, 1H), 5.64 (d, J = 8.3 Hz, 1H), 5.82 (br s, 1H), 7.27-7.33 (m, 1H), 7.53-7.57 (bs, 1 H), 7.60-7.74 (m, 4 H), 8.13-8.27 (m, 3 H), 8.30-8.35 (br s, 1H).

EXAMPLE 33

Synthesis of compound #824 (Table 8)



- A. Compound 33a (~0.55 mmol) was dissolved in CH_2Cl_2 (100 mL) and the solution was degassed carefully before a sample of Hoveyda's catalyst (17 mg, 0.028 mmol, 0.05 eq.) was added. The solution was then stirred under reflux for 5 h. The reaction mixture was concentrated and purified by flash column chromatography, using a solvent gradient of $\text{CH}_2\text{Cl}_2/\text{EtOAc}$ (from 3:2 to 2:3 ratio), to give compound 33b in 72% yield (194 mg).
- 10 B. To a solution of compound 33b (70 mg, 0.142 mmol), 2-ethoxy-4-hydroxy-7-methoxyquinoline 3c (63 mg, 0.284 mmol, 2 eq.) and Ph_3P (186 mg, 0.71 mmol, 5 eq.) in anhydrous THF (15 mL) at 0°C , DIAD (140 μL , 0.71 mmol, 5 eq.) was added slowly over a period of 20 min. The reaction mixture was allowed to warm-up to RT and to stir at RT for 2.5 h. Subsequently, the THF was evaporated under vacuum
- 15 and the crude product was purified by flash column chromatography, using a

solvent gradient of hexane/EtOAc (from 7:3 to 1:1 ratio). Pure compound 33c was isolated in 73% yield (72 mg).

C. Compound 33c (72 mg, 0.104 mmol) was mixed with CH_2Cl_2 (5 mL) and 4M HCl in dioxane (5 mL) and the mixture was allowed to stir at RT for 1.5 h in order to
5 cleave the Boc protecting group and obtain the HCl salt of intermediate 33d. The reaction crude reaction mixture was evaporated to dryness under vacuum, dried under vacuum to assure the removal of all HCl and used in the next step without purification.

D. To a solution of cyclopentanol (29 μL , 0.32 mmol) in THF (10 mL), a solution
10 of phosgene in toluene (1.93 M, 274 μL , 0.528 mmol) was added dropwise and the mixture was stirred at R.T. for 2 h to form the cyclopentyl chloroformate reagent. After that period, approximately half of the solvent was removed by evaporation under vacuum, the remaining light yellow solution was diluted by the addition of CH_2Cl_2 (5 mL) and reconcentrated to half of its original volume, in order to assure
15 the removal of all excess phosgene. The above solution of the cyclopentyl chloroformate reagent was further diluted with THF (10 mL), cooled to 0 °C and added to the solid compound 33d (0.104 mmol) at 0 °C. Et_3N (75 μL , 0.534 mmol, 5.2 eq.) was added to the reaction mixture and stirring was continued at 0 °C for 1.5 h. The solvents were removed under vacuum and the crude material purified by flash
20 column chromatography, using EtOAc/hexane (1:1) as the eluent, to obtain compound 33e in almost quantitative yield (75 mg).

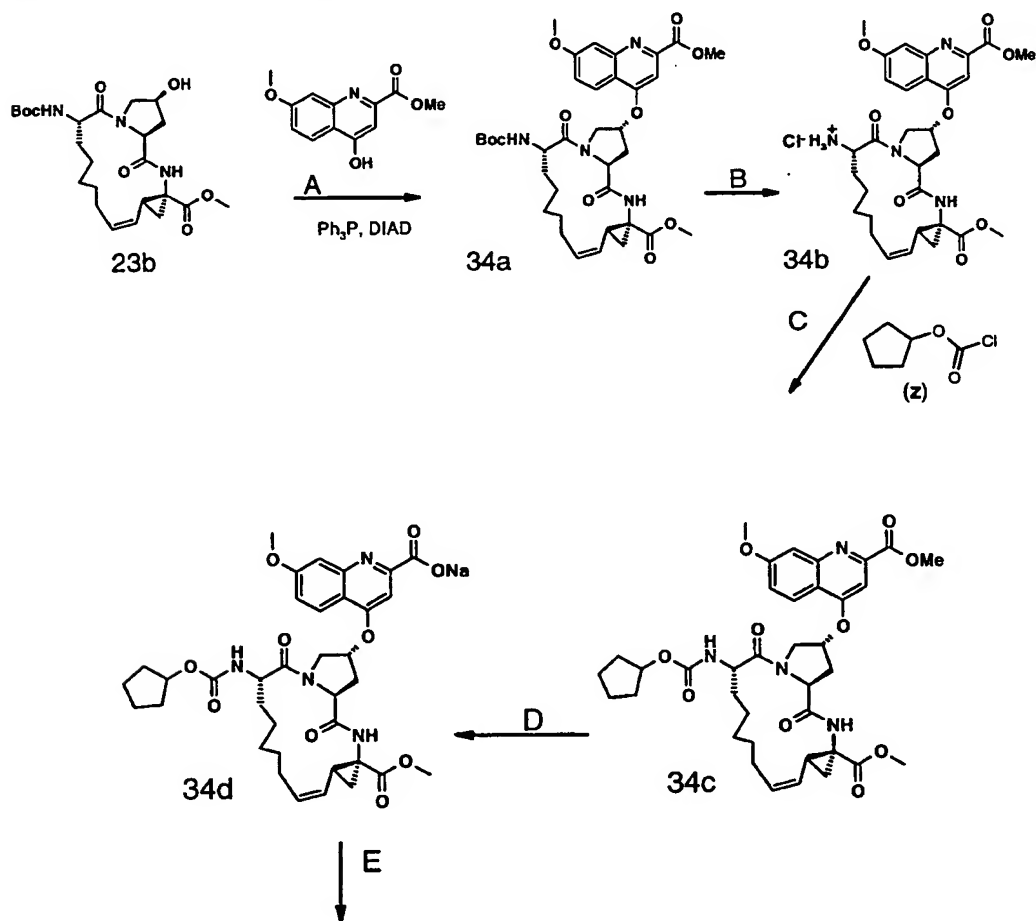
E. Hydrolysis of the methyl ester was achieved by reacting compound 33e (75 mg, 0.11 mmol) with $\text{LiOH}\cdot\text{H}_2\text{O}$ (35 mg, 0.84 mmol, 8 eq.) in a solvent mixture of THF/MeOH/ H_2O (2:2:1 ratio, 7.5 mL) at 50 °C for 2.5 h. Upon completion of the
25 hydrolysis, the mixture was acidified to pH=4.5 and the solvents were evaporated to dryness under vacuum. The crude product was purified by C18 reversed phase preparative HPLC, using a solvent gradient of H_2O to 58% aqueous CH_3CN (with 0.06%TFA), to obtain inhibitor #824 as a white amorphous solid (45 mg, 65% yield).
 ^1H NMR of the Na^+ salt of #824 (DMSO, 400 MHz): δ 0.88 (d, $J=6.7$ Hz, 3H), 0.95-1.70
30 (overlapping resonances, 17H), 1.37 (t, $J=7$ Hz, 3H), 2.00-2.10 (m, 1H), 2.10-2.33 (m,

88

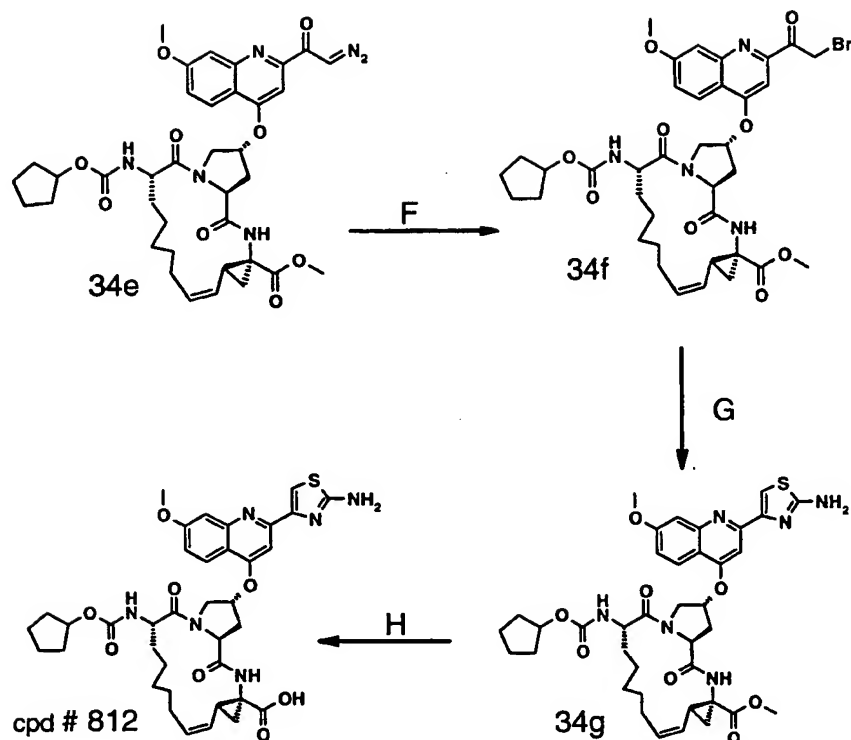
3H), 2.38-2.44 (m, 1H), 3.80-3.85 (m, 1H), 3.85 (s, 3H), 4.02-4.08 (m, 1H), 4.42 (q, J=7 Hz, 2H), 4.35-4.44 (m, 1H), 4.50 (d, J=10.8 Hz, 1H), 4.63 (bs, 1H), 5.28 (dd, J=9.5 Hz, 1H), 5.38 (bs, 1H), 5.42-5.49 (m, 1H), 6.37 (s, 1H), 6.87 (dd, J=8.9 & 2.2 Hz, 1H), 7.07 (d, J=2.2 Hz, 1H), 7.28 (d, J=7.0 Hz, 1H), 7.90 (d, J=8.9 Hz, 1H), 8.57 (s, 1H).

5 EXAMPLE 34

Synthesis of compound #812 (Table 8)



89



- A. To a solution of the macrocyclic intermediate 23b (13.05 g, 27.2 mmol, 1.0 eq.), Ph_3P (14.28 g, 54.4 mmol, 2.0 eq) and 2-carboxymethoxy-4-hydroxy-7-methoxyquinoline (WO 00/09543 & WO 00/09558) (6.67 g, 28.6 mmol, 1.05 eq) in
- 5 THF (450 mL) at 0°C , DIAD (10.75 mL, 54.6 mmol, 2.0 eq) was added dropwise over a period of 15 min. The ice bath was then removed and the reaction mixture was stirred at RT for 3 h. After the complete conversion of starting material to products, the solvent was evaporated under vacuum, the remaining mixture diluted with
- 10 EtOAc, washed with saturated NaHCO_3 (2x) and brine (1x), the organic layer was dried over anhydrous MgSO_4 , filtered and evaporated to dryness. Pure compound 34a was obtained after flash column chromatography; the column was eluted first with hexane/EtOAc (50:50), followed by CHCl_3 /EtOAc (95:5) to remove Ph_3PO and DIAD byproducts and elution of the impurities was monitored by TLC. Finally, the desired product 34a was eluted from the column with CHCl_3 /EtOAc (70:30).
- 15 Usually, the chromatography step had to be repeated 2-3 times before compound 34a could be isolated in high purity as a white solid with an overall yield of 68%

(12.8 g, 99.5% pure by HPLC).

- B. To a solution of the Boc-protected intermediate **34a** (1.567g) in CH_2Cl_2 (15 mL), 4N HCl in dioxane (12 mL) was added and the reaction mixture was stirred at RT for 1 h. [In the event that a thick gel would form half way through the reaction
5 period, an additional 10 mL CH_2Cl_2 was added.] Upon completion of the deprotection the solvents were evaporate to dryness to obtain a yellow solid and a paste like material. The mixture was redissolved in approximately 5% MeOH in CH_2Cl_2 and re-evaporated to dryness under vacuum to obtain compound **34b** as a yellow solid, which was used in the next step without any purification.
- 10 C. To a solution of cyclopentanol (614 μL , 6.76 mmol) in THF (15 mL), a solution of phosgene in toluene (1.93 M, 5.96 mL, 11.502 mmol) was added dropwise and the mixture was stirred at R.T. for 2 h to form the cyclopentyl chloroformate reagent (**z**). After that period, approximately half of the solvent was removed by evaporation under vacuum, the remaining light yellow solution was diluted by the
15 addition of CH_2Cl_2 (5 mL) and concentrated to half of its original volume, in order to assure the removal of all excess phosgene. The above solution of the cyclopentyl chloroformate reagent was further diluted with THF (15 mL) and added to the amine-2HCl salt **34b**. The mixture was cooled to 0°C in an ice bath, the pH was adjusted to ~8.5-9 with the addition of Et_3N (added dropwise) and the reaction
20 mixture was stirred at 0°C for 1 h. After that period, the mixture was diluted with EtOAc, washed with water (1x), saturated NaHCO_3 (2x), H_2O (2x) and brine (1x). The organic layer was dried over anhydrous MgSO_4 , filtered and evaporated under vacuum to obtain a yellow-amber foam. Compound **34c** was obtained as a white foam after purification by flash column chromatography (using a solvent gradient
25 from 30% hexane to 20% hexane in EtOAc as the eluent) in 80% yield (1.27 g) and >93% purity.
- D. The dimethyl ester **34c** (1.17g) was dissolved in a mixture of THF/MeOH/ H_2O (20 mL, 2:1:1 ratio), and an aqueous solution of NaOH (1.8 mL, 1N, 1 eq.) was added. The reaction mixture was stirred at RT for 1 h before it was
30 evaporated to dryness to obtain the sodium salt **34d** as a white solid (~1.66 mmol).

Compound 34d was used in the next step without purification.

E. The crude sodium salt 34d (1.66 mmol) was dissolved in THF (17 mL), Et₃N was added and the mixture was cooled to 0°C in an ice bath. Isobutylchloroformate (322 µL, 2.5 mmol) was added dropwise and the mixture was stirred at 0°C for 75
5 min. After that period, diazomethane (15 mL) was added and stirring was continued at 0°C for 30 min and then at RT for an additional 1 h. Most of the solvent was evaporated to dryness under vacuum, the remaining mixture was diluted with EtOAc, washed with saturated NaHCO₃ (2x), H₂O (2x) and brine (1x), dried over anhydrous MgSO₄, filtered and evaporated to dryness to obtain compound 34e as a
10 light yellow foam (1.2g, ~1.66 mmol). The diazoketone intermediate 34e was used in the next step without purification.

F. The diazoketone 34e (1.2g, 1.66 mmol) dissolved in THF (17 mL) was cooled to 0°C in an ice bath. A solution of aqueous HBr (48%, 1.24 mL) was added dropwise and the reaction mixture was stirred at 0°C for 1 h. The mixture was then
15 diluted with EtOAc, wash with saturated NaHCO₃ (2x), H₂O (2x) and brine (1x), the organic layer was dried over anhydrous MgSO₄, filtered and evaporated to dryness to obtain the β-bromoketone intermediate 34f as a light yellow foam (~1.657 mmol).

G. To a solution of the bromoketone 34f (600 mg, 0.779 mmol) in isopropanol (5 mL), thiourea (118 mg, 1.55 mmol) was added and the reaction mixture was placed
20 in a pre-heated oil bath at 75°C where it was allowed to stir for 1 hr. The isopropanol was then removed under vacuum and the product dissolved in EtOAc (100 mL). The solution was washed with saturated NaHCO₃ and brine, the organic layer was dried over anhydrous Na₂SO₄, filtered and evaporated to afford the crude product 34g (522 mg) as a red-brown solid. This material was used in the final step
25 without any further purification.

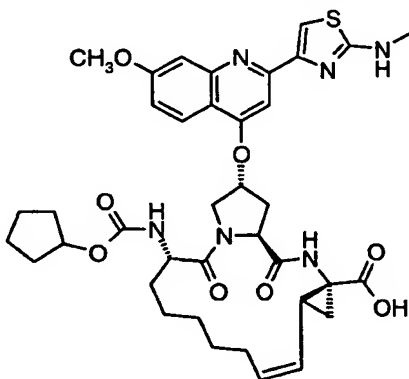
H. The crude methyl ester 34g (122 mg, 0.163 mmol) was dissolved in a solution of THF/MeOH/H₂O (2:1:1 ratio, 4 mL) and saponified using LiOH•H₂O (89 mg, 2.14 mmol). The hydrolysis reaction was carried out over a 12-15 h period at RT. The

solvents were then removed under vacuum and the crude product purified by C18 reversed phase HPLC, using a solvent gradient from 10% CH₃CN in H₂O to 100% CH₃CN, to afford the HCV protease inhibitor #812 as a yellow solid (24 mg, 20% overall yield for the conversion of intermediate 34f to inhibitor #812).

- 5 ¹H NMR (400 MHz, DMSO-d₆) δ 8.63 (s, 1H), 8.26-8.15 (m, 2H), 7.79 (bs, 1H), 7.72 (bs, 1H), 7.50 (bs, 2H), 7.33-7.25 (m, 2H), 5.77 (bs, 1H), 5.52 (dd, J = 8.3 Hz, 1H), 5.27 (dd, J = 9.2 Hz, 1H), 4.64 (d, J = 10.8 Hz, 1H), 4.50 (dd, J = 8.3 Hz, 1H), 4.39-4.31 (m, 1H), 4.08-3.99 (m, 2H), 3.94 (s, 3H), 3.87 (d, J = 9.5 Hz, 2H), 2.65-2.53 (m, 2H), 2.46-2.36 (m, 2H), 2.20-2.12 (dd, J = 8.6 Hz, 1H), 1.80-1.64 (m, 2H), 1.63-1.06 (m, 14H). MS; es⁺: 733.2 (M + H)⁺, es⁻: 731.2 (M - H)⁻.
- 10

EXAMPLE 34A

Using the same procedure as described in example 34 but reacting bromoketone 34f with commercially available *N*-methylthiourea gave # 811 (Table 8)

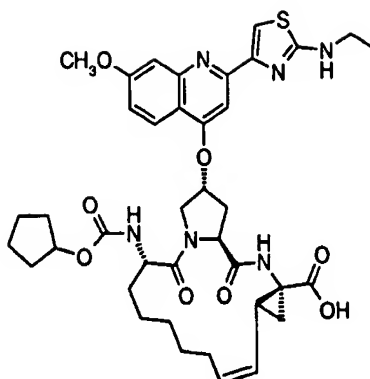


- 15 ¹H NMR (400 MHz, DMSO-d₆): δ 8.63 (s, 1H), 8.20 (s, 1H), 8.18 (s, 1H), 8.12-7.93 (m, 1H), 7.88-7.69 (m, 2H), 7.32-7.24 (m, 2H), 5.82-5.75 (m, 1H), 5.52 (ddd, J= 8.1 Hz, 1H), 5.28 (dd, J= 9.9 Hz, 1H), 4.67-4.61 (m, 1H), 4.51 (dd, J= 8.8 Hz, 1H), 4.44-4.37 (m, 1H), 4.08-4.00 (m, 1H), 3.96 (s, 3H), 3.89 (m, 1H), 3.04 (d, J= 4.1 Hz, 3H), 2.65-2.37 (m, 3H), 2.16 (m, 1H), 1.77-1.65 (m, 2H), 1.63-1.11 (m, 17H). MS; es⁺: 747.2 (M +
- 20 H)⁺, es⁻: 745.3 (M - H)⁻.

EXAMPLE 34B

Using the same procedure as described in example 34 but reacting bromoketone 34f with commercially available *N*-ethylthiourea gave # 810 (Table 8)

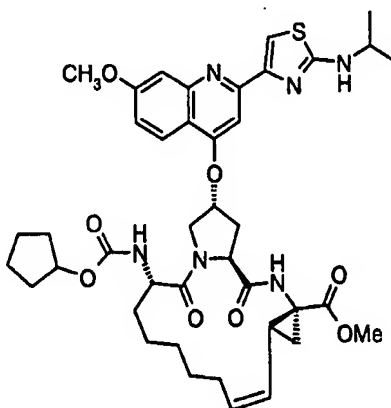
93



¹H NMR (400 MHz, DMSO-d₆): δ 8.63 (s, 1H), 8.27 (bs, 1H), 8.20 (d, J = 9.0 Hz, 1H), 8.13-8.07 (m, 1H), 7.86 (bs, 1H), 7.78 (s, 1H), 7.33-7.25 (m, 2H), 5.81 (bs, 1H), 5.54 (dd, J = 8.8 Hz, 1H), 5.28 (dd, J = 9.7 Hz, 1H), 4.65 (d, J = 12.4 Hz, 1H), 4.51 (dd, J = 8.8 Hz, 1H), 4.38 (bs, 1H), 4.03 (m, 1H), 3.97 (s, 3H), 3.92-3.87 (m, 1H), 3.54-3.46 (m, 2H), 2.68-2.65 (m, 2H), 2.47-2.38 (m, 1H), 2.15 (dd, J = 8.6 Hz, 1H), 1.78-1.65 (m, 2H), 1.60-1.12 (m, 17H), 1.25 (t, J = 7.3 Hz, 3H). MS; es⁺: 783.2 (M + Na)⁺, es⁻: 761.2 (M + H)⁺.

EXAMPLE 34C

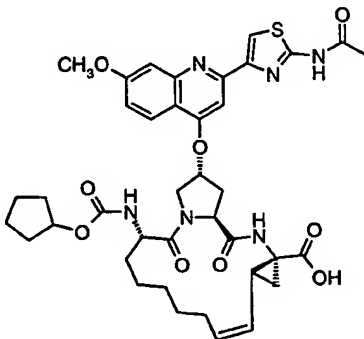
- 10 Using the same procedure as described in example 34 but reacting bromoketone 34f with commercially available *N*-iso-propylthiourea gave # 822



¹H NMR (400 MHz, DMSO-d₆): δ 8.63 (s, 1H), 8.33-8.23 (bs, 1H), 8.21 (d, J = 9.2 Hz, 1H), 8.04 (d, J = 8.3 Hz, 1H), 7.86 (bs, 1H), 7.77 (s, 1H), 7.35-7.23 (m, 2H), 5.81 (bs, 1H), 5.52 (dd, J = 8.5 Hz, 1H), 5.27 (dd, J = 9.2 Hz, 1H), 4.65 (d, J = 11.8 Hz, 1H), 4.51 (dd, J = 7.6 Hz, 1H), 4.37 (bs, 1H), 4.15 (bs, 1H), 4.07-3.98 (m, 2H), 3.97 (s, 3H), 3.88 (d, J = 8.9 Hz, 1H), 2.60-2.53 (m, 2H), 2.47-2.37 (m, 2H), 2.19-2.10 (dd, J = 9.2 Hz, 1H), 1.80-1.64 (m, 2H), 1.63-1.29 (m, 13H), 1.27 and 1.25 (2 x d, J = 6.5 Hz, 6H), 1.23-1.09

EXAMPLE 34D

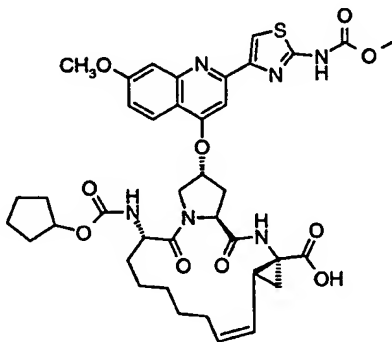
Using the same procedure as described in example 34 but reacting bromoketone 34f with commercially available *N*-acetylthiourea gave # 809



- 5 ¹H NMR (400 MHz, DMSO-d₆): δ 8.62 (s, 1H), 8.30 (bs, 1H), 8.17 (d, J = 8.9 Hz, 1H), 7.62 (bs, 1H), 7.52 (bs, 1H), 7.28 (d, J = 6.4 Hz, 1H), 7.21 (bs, 1H), 5.63 (bs, 1H), 5.54 (dd, J = 8.1 Hz, 1H), 5.28 (dd, J = 9.5 Hz, 1H), 4.62 (d, J = 12.1 Hz, 1H), 4.56-4.46 (m, 2H), 4.11-4.04 (m, 1H), 3.95 (s, 3H), 3.93-3.88 (m, 1H), 2.62-2.54 (m, 1H), 2.45-2.36 (m, 1H), 2.22 (s, 3H), 2.21-2.13 (m, 1H), 1.79-1.69 (m, 2H), 1.65-1.30 (m, 16H), 1.26-1.12 (m, 2H). MS; es⁺: 775.3 (M + H)⁺, es⁻: 773.3 (M - H)⁻.
- 10

EXAMPLE 34E

- To a stirred solution of the 2-amino-4-thiazolyl intermediate 34g (0.24 g, 0.32 mmol) in CH₂Cl₂ (5 mL) at RT was added DIPEA (0.55 mL, 3.18 mmol, 10 eq) and methyl chloroformate (0.13 mL, 1.6 mmol, 5 eq). The reaction mixture was stirred for 6.5 h
- 15 before being concentrated under vacuum. The crude isolated material was then hydrolyzed to the desired carboxylic acid as described in Example 34 to yield compound # 818

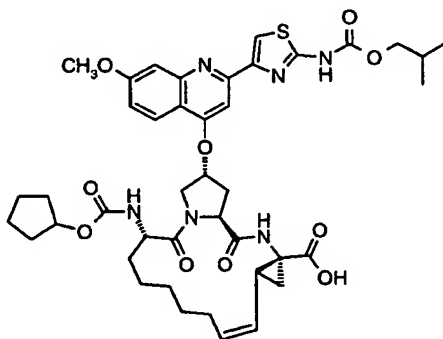


- ¹H NMR (400 MHz, DMSO-d₆): δ 8.61 (s, 1H), 8.21-8.07 (m, 2H), 7.61-7.38 (m, 2H), 7.26 (d, J = 6.4 Hz, 1H), 7.19-7.10 (m, 1H), 5.60-5.47 (m, 2H), 5.27 (dd, J = 9.2 Hz,
- 20

1H), 4.63-4.53 (m, 1H), 4.47 (d, J = 7.9 Hz, 1H), 4.13-4.04 (m, 1H), 3.93 (s, 3H), 3.92-3.87 (m, 2H), 3.79 (s, 3H), 2.42-2.30 (m, 2H), 2.17 (dd, J = 9.2 Hz, 1H), 1.81-1.68 (m, 2H), 1.63-1.29 (m, 16H), 1.23-1.10 (m, 2H). MS; es^+ : 791.1 (M + H) $^+$, es^- : 789.1 (M - H) $^-$.

EXAMPLE 34F

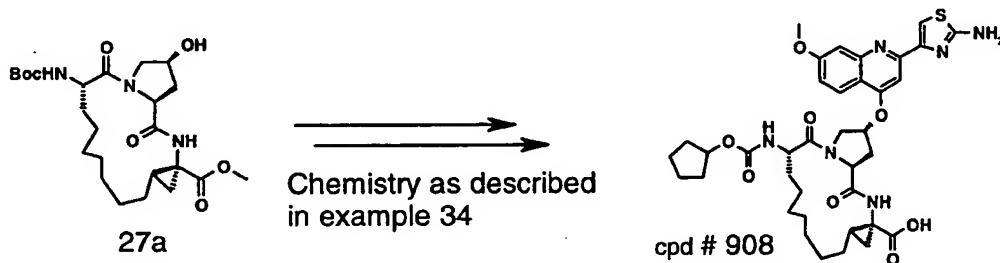
- 5 Following the conditions described above in example 34E, but using isobutyl chloroformate, gave the analogous substituted carbamate intermediate. The crude isolated material was then hydrolyzed to the desired compound # 819



- 1H NMR (400 MHz, DMSO- d_6): δ 8.62 (s, 1H), 8.47-8.27 (bs, 1H), 8.18 (d, J = 8.6 Hz, 1H), 7.69-7.60 (m, 1H), 7.60-7.51 (m, 1H), 7.28 (d, J = 6.7 Hz, 1H), 7.28-7.19 (m, 1H), 5.70-5.60 (m, 1H), 5.52 (dd, J = 8.3 Hz, 1H), 5.27 (dd, J = 9.8 Hz, 1H), 4.63 (d, J = 11.8 Hz, 1H), 4.53-4.44 (m, 2H), 4.10-3.99 (m, 1H), 4.04 (d, J = 6.7 Hz, 2H), 3.95 (s, 3H), 3.94-3.87 (m, 1H), 2.65-2.53 (m, 1H), 2.46-2.34 (m, 1H), 2.16 (dd, J = 8.1 Hz, 1H), 2.03-1.91 (m, 1H), 1.79-1.09 (m, 20H), 0.95 (d, J = 6.7 Hz, 6H). MS; es^+ : 833.2 (M + H) $^+$, es^- : 831.2 (M - H) $^-$.

EXAMPLE 35**Synthesis of compound #908**

Starting with derivative 27a and using the same chemistry as described in example 34, the following saturated macrocycle, compound # 908 (Table 9) was obtained.



20

1H NMR (400 MHz, DMSO- d_6): δ 8.47 (s, 1H), 8.16 (d, J = 10 Hz, 1H), 8.15-8.07 (m,

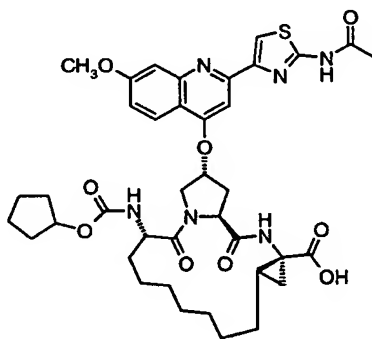
96

1H), 7.82-7.63 (m, 2H), 7.53-7.43 (m, 2H), 7.33-7.22 (m, 1H), 7.13 (d, $J = 7$ Hz, 1H), 5.77-5.65 (m, 1H), 4.62-4.52 (m, 2H), 4.50-4.4 (m, 1H), 4.20-4.10 (m, 1H), 3.94 (s, 3H), 3.89-3.83 (m, 1H), 2.59-2.53 (m, 1H), 2.48-2.40 (m, 1H), 1.79-1.0 (m, 25H);). MS; es^+ : 735.2 ($M + H$) $^+$, es^- : 733.2 ($M - H$) $^-$.

5 EXAMPLE 35A

Synthesis of compound #909

Using the same procedure as described in example 35 but using available *N*-acetylthiourea gave compound # 909 (Table 9).

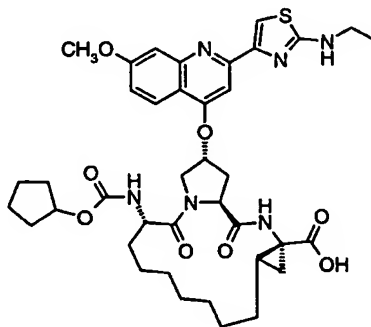


- 10 1H NMR (400 MHz, DMSO- d_6): δ 8.53-8.41 (m, 2H), 8.20 (d, $J = 9.2$ Hz, 1H), 7.68 (bs, 1H), 7.68 (bs, 1H), 7.27 (dd, $J = 9.2$ Hz, 1H), 7.15 (d, $J = 6.4$ Hz, 1H), 5.67 (bs, 1H), 4.65-4.50 (m, 3H), 4.44-4.37 (m, 1H), 4.21-4.13 (m, 1H), 3.96 (s, 3H), 3.99-3.86 (m, 1H), 2.62-2.39 (m, 2H), 2.24 (s, 3H), 1.78-1.67 (m, 3H), 1.67-1.01 (m, 22H). MS; es^+ : 798.0 ($M + Na$) $^+$, es^- : 777.0 ($M + H$) $^+$.

15 EXAMPLE 35B

Synthesis of compound #910

Using the same procedure as described in example 35 but using available *N*-ethylthiourea gave compound # 910 (Table 9).



- 20 1H NMR (400 MHz, DMSO- d_6): δ 8.47 (s, 1H), 8.29 (bs, 1H), 8.20 (d, $J = 9.2$ Hz, 1H),

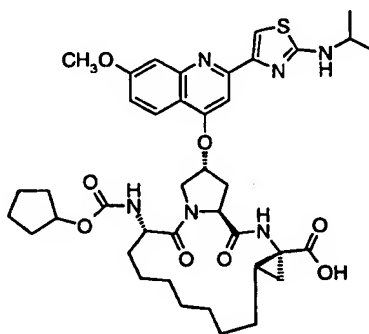
97

8.09 (bs, 1H), 7.87 (s, 1H), 7.77 (s, 1H), 7.32 (dd, $J = 9.2$ Hz, 1H), 7.14 (dd, $J = 6.7$ Hz, 1H), 5.78 (bs, 1H), 4.58 (dd, $J = 8.1$ Hz, 2H), 4.43 (bs, 1H), 4.18-4.12 (m, 1H), 3.97 (s, 3H), 3.87 (d, $J = 8.9$ Hz, 1H), 3.55-3.46 (m, 2H), 2.63-2.53 (m, 1H), 2.47-2.41 (m, 1H), 1.78-1.00 (m, 25H), 1.25 (t, $J = 7.3$ Hz, 3H). MS; m/z : 763.1 ($M + H$)⁺, m/z : 761.1 ($M - H$)⁻.

5

EXAMPLE 35C**Synthesis of compound #911**

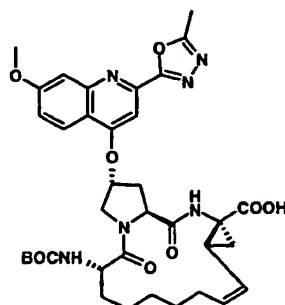
Using the same procedure as described in example 35 but using available *N*-isopropylthiourea gave compound # 911 (Table 9).



10

¹H NMR (400 MHz, DMSO-*d*₆): δ 8.47 (s, 1H), 8.29-8.19 (m, 1H), 8.19 (d, $J = 9.2$ Hz, 1H), 8.09-8.0 (m, 1H), 7.83 (bs, 1H), 7.74 (bs, 1H), 7.31 (d, $J = 8$ Hz, 1H), 7.14 (d, $J = 6.4$ Hz, 1H), 5.76 (bs, 1H), 4.64-4.53 (m, 2H), 4.44 (bs, 1H), 4.22-4.09 (m, 3H), 3.97 (s, 3H), 3.87 (d, $J = 8.6$ Hz, 1H), 2.63-2.58 (m, 1H), 2.46-2.41 (m, 1H), 1.79-1.10 (m, 24H), 1.27 and 1.26 (2 x d, $J = 6.5$ Hz, 6H). MS; m/z : 777.0 ($M + H$)⁺, m/z : 775.0 ($M - H$)⁻.

15

EXAMPLE 36**Synthesis of compound #716**

¹H NMR (400 MHz, DMSO-*d*₆): δ (ppm): 8.62 (s, 1H), 8.13 (d, $J=9.2$ Hz, 1H), 7.64-7.54 (m, 2H), 7.47 (d, $J=2.6$ Hz, 1H), 7.16 (dd, $J=9.2, 2.2$ Hz, 1H), 7.03 (d, $J=6.0$ Hz, 1H),

20

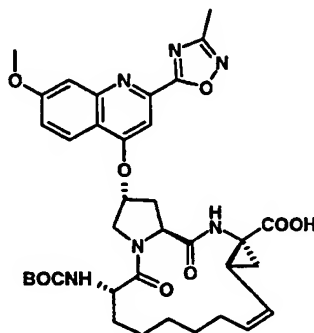
98

5.63 (s, 1H), 5.52 (q, J=9.9 Hz, 1H), 5.26 (t, J=8.9 Hz, 1H), 4.62 (d, J=11.45, 1H), 4.45 (dd, J=9.2, 8.27 Hz, 1H), 4.02 (m, 1H), 3.93 (s, 3H), 3.7 (dd, J=7.6, 1.0 Hz, 1H), 2.66 (s, 3H), 2.55-2.65 (m, 1H), 2.35-2.45 (m, 1H), 2.17 (q, J=8.6 Hz, 1H), 1.65-1.75 (m, 2H), 1.5-1.35 (m, 7H), 1.15 (s, 9H).

5 MS : 705. (M+1), 703 (M-1)

EXAMPLE 37

Synthesis of compound #717

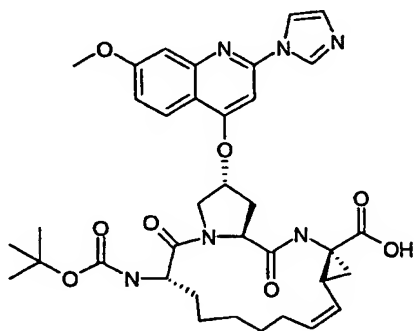


¹H NMR (400 MHz, DMSO-d₆): δ (ppm): 8.62 (s, 1H), 8.15 (d, J=8.9 Hz, 1H), 7.62 (s, 1H), 7.49 (s, 1H), 7.19 (dd, J=9.2, 2.2 Hz, 1H), 7.02 (d, J=5.4 Hz, 1H), 5.64 (s, 1H), 5.52 (q, J=9.9 Hz, 1H), 5.26 (t, J=9.2 Hz, 1H), 4.63 (d, J=11.44, 1H), 4.45 (t, J=9.2 Hz, 1H), 3.94 (s, 3H), 3.9-3.8 (m, 1H), 2.7-2.55 (m, 1H), 2.4-2.3 (m, 1H), 2.18 (q, J=8.9 Hz, 1H), 1.75-1.65 (m, 2H), 1.5-1.2 (m, 7H), 1.14 (s, 9H).

MS : 705. (M+1), 703 (M-1).

15 EXAMPLE 38

Synthesis of compound #718



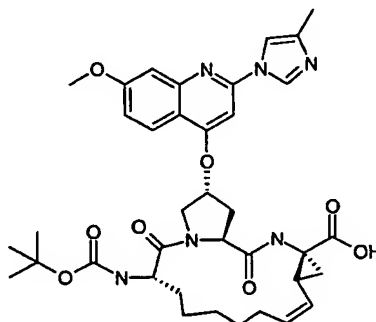
¹H NMR (400 MHz, DMSO-d₆): δ (ppm): 9.55 (s, 1H), 8.63 (s, 1H), 8.43 (s, 1H), 8.13 (d, J = 9.2 Hz, 1H), 7.66 (s, 1H), 7.46 (s, 1H), 7.32 (d, J = 2.6 Hz, 1H), 7.10-7.07 (m, 2H),

5.64-5.54 (m, 1H), 5.59-5.48 (m, 1H), 5.33-5.23 (m, 1H), 4.73-4.61 (m, 1H), 4.45 (dd, J = 7.5, 9.1 Hz, 1H), 4.09-4.00 (m, 1H), 3.92 (s, 3H), 3.93-3.83 (m, 1H), 2.67-2.55 (m, 2H), 2.53-2.43 (m, 1H), 2.42-2.31 (m, 1H), 2.23-2.12 (m, 1H), 1.81-1.66 (m, 2H), 1.52-1.42 (m, 2H), 1.42-1.25 (m, 6H), 1.21 (s, 9H).

5 MS: 689.3 (M+1), 687.3 (M-1)

EXAMPLE 39

Synthesis of compound #722

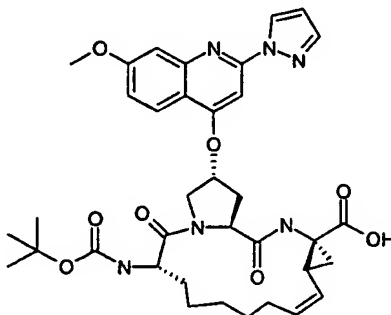


¹H NMR (400 MHz, DMSO-d₆): δ (ppm): 9.70 (s, 1H), 8.64 (s, 1H), 8.26 (s, 1H), 8.14 (d, J = 9.2 Hz, 1H), 7.45 (s, 1H), 7.30 (d, J = 2.5 Hz, 1H), 7.14-7.06 (m, 2H), 5.60-5.54 (m, 1H), 5.58-5.48 (m, 1H), 5.31-5.23 (m, 1H), 4.71-4.62 (m, 1H), 4.49-4.40 (m, 1H), 4.08-3.99 (m, 1H), 3.92 (s, 3H), 3.92-3.84 (m, 1H), 2.69-2.54 (m, 2H), 2.53-2.46 (m, 1H), 2.42-2.31 (m, 1H), 2.37 (s, 3H), 2.22-2.13 (m, 1H), 1.81-1.64 (m, 2H), 1.54-1.42 (m, 2H), 1.42-1.27 (m, 6H), 1.22 (s, 9H).

15 MS: 703.3 (M+1), 701.3 (M-1)

EXAMPLE 40

Synthesis of compound #733



¹H NMR (400 MHz, DMSO-d₆): δ (ppm): 8.75 (m, 1H), 8.62 (s, 1H), 8.06 (d, J=9.2 Hz, 1H), 7.88-7.87 (m, 1H), 7.48 (s, 1H), 7.28 (d, J=2.6 Hz, 1H), 7.05-7.00 (m, 2H), 6.64-6.63

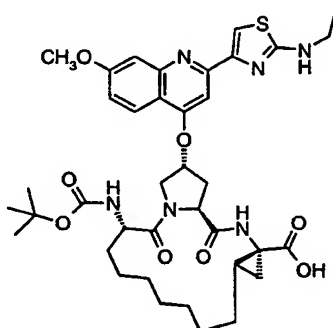
100

(m, 1H), 5.62-5.58 (m, 1H), 5.55-5.49 (m, 1H), 5.28-5.24 (m, 1H), 4.64-4.61 (m, 1H), 4.48-4.44 (m, 1H), 4.07-4.03 (m, 1H), 3.91 (s, 3H), 3.92-3.85 (m, 1H), 2.67-2.54 (m, 2H), 2.53-2.45 (m, 1H), 2.41-2.34 (m, 1H), 2.20-2.14 (m, 1H), 1.75-1.69 (m, 2H), 1.50-1.43 (m, 2H), 1.41-1.32 (m, 6H), 1.17 (s, 9H).

5 MS : 689.3 (M+1), 687.2 (M-1)

EXAMPLE 41

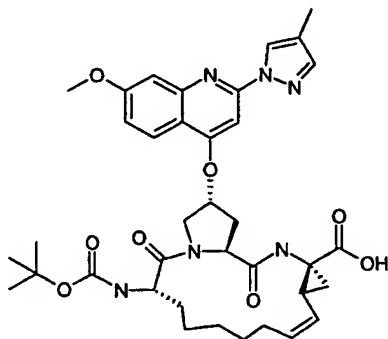
Synthesis of compound # 703



¹H NMR (400 MHz, DMSO-d₆): δ 8.50 (s, 1H), 8.19 (s, 1H), 8.17 (s, 1H), 8.11-8.00 (m, 1H), 7.88-7.77 (m, 1H), 7.73 (s, 1H), 7.25 (d, J = 8.6 Hz, 1H), 6.93 (d, J = 6 Hz, 1H), 5.89-5.68 (m, 1H), 4.62 (d, J = 11 Hz, 1H), 4.53 (dd, J = 8.3 Hz, 1H), 4.16-4.07 (m, 1H), 3.96 (s, 3H), 3.88 (bd, J = 9.5 Hz, 1H), 3.53-3.43 (m, 2H), 2.63-2.51 (m, 1H), 2.46-2.36 (m, 1H), 1.81-1.62 (m, 2H), 1.60-1.01 (m, 15H), 1.24 (t, J = 7.4 Hz, 3H), 1.17 (s, 9H).
MS; es⁺: 751.1(M + H)⁺, es⁻: 749.1- (M - H)⁻.

15 EXAMPLE 42

Synthesis of compound #734



¹H NMR (400 MHz, DMSO-d₆): δ (ppm): 8.62 (s, 1H), 8.54 (s, 1H), 8.04 (d, J=9.2 Hz, 1H), 7.70 (s, 1H), 7.43 (s, 1H), 7.24 (d, J=2.6Hz, 1H), 7.05-6.98 (m, 2H), 5.57-5.54 (m,

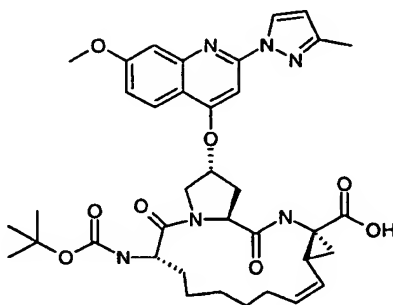
101

1H), 5.55-5.48 (m, 1H), 5.28-5.24 (m, 1H), 4.63-4.59 (m, 1H), 4.47-4.43 (m, 1H), 4.13-3.99 (m, 1H), 3.90 (s, 3H), 3.92-3.83 (m, 1H), 2.67-2.55 (m, 2H), 2.53-2.46 (m, 1H), 2.43-2.31 (m, 1H), 2.22-2.15 (m, 1H), 2.15 (3H), 1.75-1.70 (m, 2H), 1.51-1.42 (m, 2H), 1.41-1.28 (m, 6H), 1.17 (s, 9H).

5 MS : 703.2 (M+1), 701.3 (M-1)

EXAMPLE 43

Synthesis of compound #738

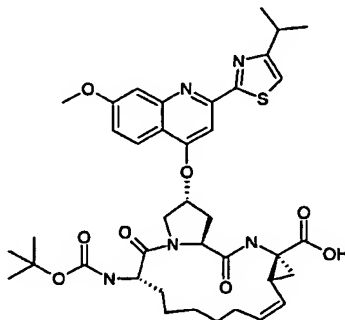


¹H NMR (400 MHz, DMSO-d₆): δ (ppm): 8.64 (d, J = 2.5 Hz, 1H), 8.62 (s, 1H), 8.04 (d, J = 9.2 Hz, 1H), 7.39 (s, 1H), 7.24 (d, J = 2.5 Hz, 1H), 7.04 (d, J = 6.0 Hz, 1H), 6.99 (dd, J = 2.2, 9.2 Hz, 1H), 6.43 (d, J = 2.2 Hz, 1H), 5.62-5.57 (m, 1H), 5.56-5.47 (m, 1H), 5.31-5.22 (m, 1H), 4.65-4.56 (m, 1H), 4.45 (dd, J = 7.6, 8.9 Hz, 1H), 4.07-4.00 (m, 1H), 3.90 (s, 3H), 3.88-3.84 (m, 1H), 2.68-2.56 (m, 2H), 2.54-2.43 (m, 1H), 2.42-2.31 (m, 1H), 2.34 (s, 3H), 2.24-2.14 (m, 1H), 1.80-1.64 (m, 2H), 1.52-1.43 (m, 2H), 1.43-1.27 (m, 6H), 1.18 (s, 9H).

MS: 703.2 (M+1), 701.2 (M-1)

EXAMPLE 44

Synthesis of compound #725



20 ¹H NMR (400 MHz, DMSO-d₆): δ (ppm): 8.62 (s, 1H), 8.10 (d, J=9.2 Hz, 1H), 7.57 (s,

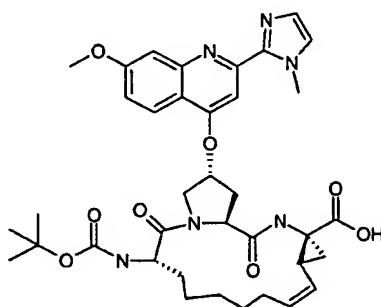
102

1H), 7.49 (s, 1H), 7.35 (d, J=2.2 Hz, 1H), 7.09-7.03 (m, 2H), 5.65-5.61 (m, 1H), 5.55-5.49 (m, 1H), 5.28-5.24 (m, 1H), 4.62-4.57 (m, 1H), 4.49-4.45 (m, 1H), 4.08-4.01 (m, 1H), 3.93 (s, 3H), 3.92-3.86 (m, 1H), 3.20-3.14 (m, 1H), 2.65-2.56 (s, 1H), 2.53-2.47 (m, 1H) 2.42-2.35 (m, 1H), 2.22-2.15 (m, 1H), 1.79-1.68 (m, 2H), 1.50-1.43 (m, 2H), 1.41-1.28 (m, 12H), 1.18 (s, 9H).

MS : 748.2 (M+1), 746.2 (M-1)

EXAMPLE 45

Synthesis of compound #726

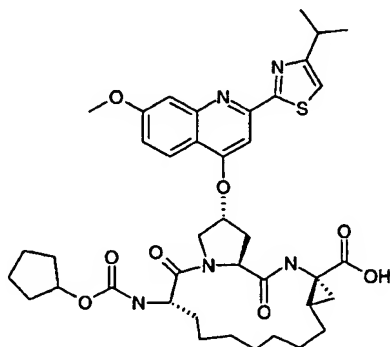


¹H NMR (400 MHz, DMSO-d₆): δ (ppm): 8.64 (s, 1H), 8.10 (d, J=9.5 Hz, 1H), 7.83-7.76 (m, 2H), 7.60 (s, 1H), 7.44-7.42 (m, 1H), 7.18-7.01 (m, 2H), 5.56-5.49 (m, 2H), 5.29-5.24 (m, 1H), 4.66-4.63 (m, 1H), 4.47-4.42 (m, 1H), 4.28 (s, 3H), 4.06-4.02 (m, 2H), 3.94 (s, 3H), 3.93-3.86 (m, 1H), 2.66-2.55 (m, 2H), 2.42-2.31 (m, 2H), 2.22-2.14 (m, 1H), 1.79-1.65 (m, 2H), 1.52-1.27 (m, 7H), 1.22 (s, 9H).

MS : 703.2 (M+1), 701.3 (M-1)

EXAMPLE 46

Synthesis of compound #906

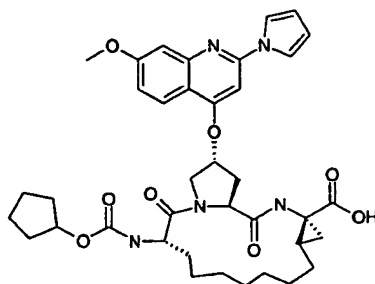


¹H NMR (400 MHz, DMSO-d₆): δ (ppm): 8.46 (s, 1H), 8.06 (d, J=9.2 Hz, 1H), 7.57 (s, 1H), 7.49 (s, 1H), 7.34 (m, 1H), 7.14-7.05 (m, 2H), 5.63-5.58 (m, 1H), 4.66-4.61 (m, 1H),

103

4.54-4.44 (m, 2H), 4.23-4.18 (m, 1H) 3.93 (s, 3H), 3.92-3.88 (m, 1H), 3.21-3.14 (m, 1H),
2.44-2.33 (m, 1H), 1.35 (d, J=7Hz, 6H), 1.73-1.01 (m, 26H)

MS : 762.0 (M+1), 759.9 (M-1)

EXAMPLE 47**5 Synthesis of compound #907**

¹H NMR (400 MHz, DMSO-d₆): δ (ppm): 8.46 (s, 1H), 7.98 (d, J=8.9 Hz, 1H), 7.91-7.89 (m, 2H), 7.23-7.21 (m, 2H), 7.07-7.00 (m, 2H), 6.35-6.32 (m, 2H), 5.64-5.58 (m, 1H),
4.65-4.61 (m, 1H), 4.53-4.47 (m, 2H), 4.24-4.19 (m, 1H) 3.90 (s, 3H), 3.86-3.84 (m, 1H),
10 2.40-2.33 (m, 1H), 1.73-1.01 (m, 26H).

MS : 702.0 (M+1), 699.9 (M-1)

EXAMPLE 48**Full-length NS3-NS4A heterodimer protein fluorogenic assay**

The NS2-NS5B-3'non coding region was cloned by RT-PCR into the pCR®3 vector
15 (Invitrogen) using RNA extracted from the serum of an HCV genotype 1b infected individual (provided by Dr. Bernard Willems, Hôpital St-Luc, Montréal, Québec, Canada). The NS3-NS4A region (NS3-NS4AFL) was then subcloned by PCR into the pFastBac™ HTa baculovirus expression vector (Gibco/BRL). The vector sequence includes a region encoding a 28-residue N-terminal sequence which contains a
20 hexahistidine tag. The Bac-to-Bac™ baculovirus expression system (Gibco/BRL) was used to produce the recombinant baculovirus. His-NS3-NS4AFL was expressed by infecting 10⁶ Sf21 cells/mL with the recombinant baculovirus at a multiplicity of infection of 0.1-0.2 at 27°. Authentic auto-proteolysis occurs during expression to produce a non covalent and stable NS3-NS4A protein complex (referred to as full-length "FL").
25 The infected culture was harvested 48 to 64 h later by centrifugation at 4°. The cell pellet was homogenized in 50mM NaPO₄, pH 7.5, 40% glycerol (w/v),

2mM β -mercaptoethanol, in presence of a cocktail of protease inhibitors. His-NS3-NS4AFL was then extracted from the cell lysate with 1.5% NP-40, 0.5% Triton X-100, 0.5M NaCl, and a DNase treatment. After ultracentrifugation, the soluble extract was diluted 4-fold and bound on a Pharmacia Hi-Trap Ni-chelating column. The

5 His-NS3-NS4AFL was eluted in a >90% pure form (as judged by SDS-PAGE), using a 50 to 400 mM imidazole gradient. The His-NS3-NS4AFL was stored at -80° in 50 mM sodium phosphate, pH 7.5, 10% (w/v) glycerol, 0.5 M NaCl, 0.25 M imidazole, 0.1% NP-40. It was thawed on ice and diluted just prior to use.

The protease activity of His-NS3-NS4AFL was assayed in 50 mM Tris-HCl, pH 8.0,

10 0.25 M sodium citrate, 0.01% (w/v) n-dodecyl- β -D-maltoside, 1 mM TCEP. Five (5) μ M of the internally quenched substrate anthranilyl-DDIVPAbu[C(O)-O]-AMY(3-NO₂)TW-OH in presence of various concentrations of inhibitor were incubated with 1.5 nM of His-NS3-NS4AFL for 45 min at 23°. The final DMSO concentration did not exceed 5.25%. The reaction was terminated with the addition of 1M MES, pH 5.8.

15 Fluorescence of the N-terminal product was monitored on a Perkin-Elmer LS-50B fluorometer equipped with a 96-well plate reader (excitation wavelength: 325 nm; emission wavelength: 423 nm).

The % inhibition was calculated with the following equation:

$$100 - [(fluo_{inh} - fluo_{blank}) / (fluo_{ctl} - fluo_{blank}) \times 100]$$

20 A non-linear curve fit with the Hill model was applied to the inhibition-concentration data, and the 50% effective concentration (IC₅₀) was calculated by the use of SAS software (Statistical Software System; SAS Institute, Inc. Cary, N.C.).

EXAMPLE 49

Recombinant HCV NS3 protease radiometric assay

25 The substrate used for the HCV NS3 protease radiometric assay, DDIVPC-SMSYTW, is cleaved between the cysteine and the serine residues by the enzyme. The sequence DDIVPC-SMSYTW corresponds to the NS5A/NS5B natural cleavage site in which the cysteine residue in P2 has been substituted for a proline. The peptide substrate DDIVPC-SMSYTW and the tracer biotin-DDIVPC-SMS[¹²⁵I-Y]TW were incubated

30 with the recombinant NS3 protease in the absence or in the presence of inhibitors. The separation of substrate from products was performed by adding avidin-coated

agarose beads to the assay mixture followed by filtration. The amount of SMS[¹²⁵I-Y]TW product found in the filtrate (with or without inhibitor) allowed for the calculation of the percentage of substrate conversion and of the percentage of inhibition.

5 A. Reagents

Tris and Tris-HCl (UltraPure) were obtained from Life Technologies. Glycerol (UltraPure), MES and BSA were purchased from Sigma[®]. TCEP was obtained from Pierce, DMSO from Aldrich[®] and NaOH from Anachemia[®].

Assay buffer: 50 mM Tris-HCl, pH 7.5, 30% (w/v) glycerol, 2% (w/v) CHAPS, 1
10 mg/mL BSA, 1 mM TCEP (TCEP added just prior to use from a 1 M stock solution in water).

Substrate: DDIVPC-SMSYTW, 25 μ M final concentration (from a 2 mM stock solution in DMSO stored at -20°C to avoid oxidation).

Tracer: reduced mono-iodinated substrate(biotin-DDIVPC-SMS[¹²⁵I-Y]TW) (\approx 1 nM
15 final concentration).

HCV NS3 protease type 1b, 25 nM final concentration (from a stock solution in 50 mM sodium phosphate, pH 7.5, 10% glycerol, 300 mM NaCl, 5 mM DTT, 0.01% NP-40).

B. Protocol

20 The assay was performed in a 96-well polypropylene plate. Each well contained:
20 μ L substrate/tracer in assay buffer;
10 μ L \pm inhibitor in 20% DMSO/assay buffer;
10 μ L NS3 protease 1b.

Blank (no inhibitor and no enzyme) and control (no inhibitor) were also prepared on
25 the same assay plate.

The enzymatic reaction was initiated by the addition of the enzyme solution and the assay mixture was incubated for 60 min at 23°C under gentle agitation. Twenty (20) μ L of 0.025 N NaOH were added to quench the enzymatic reaction.

Twenty (20) μ L of avidin-coated agarose beads (purchased from Pierce[®]) were
30 added in a Millipore[®] MADP N65 filtration plate. The quenched assay mixture was transferred to the filtration plate, and incubated for 60 min at 23°C under gentle agitation.

The plates were filtered using a Millipore® MultiScreen Vacuum Manifold Filtration apparatus, and 40 µL of the filtrate was transferred to an opaque 96-well plate containing 60 µL of scintillation fluid per well.

The filtrates were counted on a Packard® TopCount instrument using a ¹²⁵I-liquid
5 protocol for 1 minute.

The %inhibition was calculated with the following equation:

$$100 - [(counts_{inh} - counts_{blank}) / (counts_{ctrl} - counts_{blank}) \times 100]$$

A non-linear curve fit with the Hill model was applied to the inhibition-
concentration data, and the 50% effective concentration (IC₅₀) was calculated by the
10 use of SAS software (Statistical Software System; SAS Institute, Inc., Cary, N.C.).

EXAMPLE 50

Specificity assays

The specificity of the compounds was determined against a variety of serine
proteases: human leukocyte elastase, porcine pancreatic elastase and bovine
15 pancreatic α-chymotrypsin and one cysteine protease: human liver cathepsin B. In
all cases a 96-well plate format protocol using a chromogenic substrate specific for
each enzyme was used. Each assay included a 1 h enzyme-inhibitor pre-incubation
at RT followed by addition of substrate and hydrolysis to ≈30% conversion as
measured on a UV Thermomax® microplate reader or a fluorescence Perkin-Elmer®
20 LS50B plate reader. Substrate concentrations were kept as low as possible compared
to K_M to reduce substrate competition. Compound concentrations varied from 300
to 0.06 µM depending on their potency.

The final conditions for each assay were as follows:

50mM Tris-HCl pH 8, 0.5 M Na₂SO₄, 50 mM NaCl, 0.1 mM EDTA, 3% DMSO, 0.01%
25 Tween-20 with;

[100 µM Succ-AAPF-pNA and 250 pM α-chymotrypsin], [133 µM Succ-AAA-pNA
and 8 nM porcine elastase], [133 µM Succ-AAV-pNA and 8 nM leukocyte elastase];
or

[100 mM NaHPO₄ pH 6, 1 mM EDTA, 3% DMSO, 1mM TCEP, 0.01% Tween-20, 4
30 µM Z-FR-AMC (7-amino-4-methylcoumarin) and 0.5 nM cathepsin B (the stock
enzyme was activated in buffer containing 20 mM TCEP before use)].

A representative example is summarized below for porcine pancreatic elastase:

In a polystyrene flat-bottom 96-well plate (Cellwells, Corning) were added using a Biomek liquid handler (Beckman):

40 μ L of assay buffer (50 mM Tris-HCl pH 8, 1 M Na₂SO₄, 50 mM NaCl, 0.1 mM

5 EDTA);

20 μ L of enzyme solution (50 mM Tris-HCl pH 8, 50 mM NaCl, 0.1 mM EDTA, 0.02% Tween-20, 40 nM porcine pancreatic elastase); and

20 μ L of inhibitor solution (50 mM Tris-HCl, pH 8, 50 mM NaCl, 0.1 mM EDTA, 0.02% Tween-20, 1.5 mM-0.3 μ M inhibitor, 15% v/v DMSO).

10 After 60 min pre-incubation at RT, 20 μ L of substrate solution (50 mM Tris-HCl, pH 8, 0.5 M Na₂SO₄, 50 mM NaCl, 0.1 mM EDTA, 665 μ M Succ-AAA-pNA) were added to each well and the reaction was further incubated at RT for 60 min after which time the absorbance was read on the UV Thermomax® plate reader. Rows of wells were allocated for controls (no inhibitor) and for blanks (no inhibitor and no
15 enzyme).

The sequential 2-fold dilutions of the inhibitor solution were performed on a separate plate by the liquid handler using 50 mM Tris-HCl pH 8, 50 mM NaCl, 0.1 mM EDTA, 0.02% Tween-20, 15% (v/v) DMSO. All other specificity assays were performed in a similar fashion.

20 The percentage of inhibition was calculated using the formula:

$$[1 - ((UV_{inh} - UV_{blank}) / (UV_{ctl} - UV_{blank}))] \times 100$$

A non-linear curve fit with the Hill model was applied to the inhibition-concentration data, and the 50% effective concentration (IC₅₀) was calculated by the use of SAS software (Statistical Software System; SAS Institute, Inc., Cary, N.C.).

25 EXAMPLE 51

NS3 Protease Cell-based assay

This assay is done with Huh-7 cells, a human cell line derived from a hepatoma, co-transfected with 2 DNA constructs:

one (called NS3) expressing part of the HCV non-structural polyprotein fused to the
30 tTA protein through an NS5A-NS5B cleavage site in the following order: NS3-NS4A-NS4B-NS5A-(NS5B)tTA where (NS5B) represents the 6 first amino acids of NS5B.

This polyprotein is expressed under the control of the CMV promoter, the other (called SEAP) expressing the reporter protein, secreted alkaline phosphatase (SEAP), under the regulation of a tTA-responsive promoter.

The first construct leads to the expression of a polyprotein from which the different
5 mature proteins are released through cleavage by the NS3 protease. It is believed that the mature viral proteins forms a complex at the membrane of the endoplasmic reticulum. tTA is a fusion protein, described by Gossen and Bujard (Proc. Natl. Acad. Sci. USA 89 (1992): 5547-5551), which contains a DNA-binding domain and a transcriptional activator. Release of the tTA protein requires an NS3-dependent
10 cleavage at the NS5A-NS5B cleavage site between NS5A and itself. This last cleavage allows tTA to migrate to the nucleus and transactivate the SEAP gene. Therefore, reduction of NS3 proteolytic activity leads to confinement of tTA to the cytoplasm and concomitant decrease in SEAP activity.

To control for cellular activities other than inhibition of NS3 protease which are due
15 to the compound, a parallel co-transfection is done with a construct expressing tTA alone and the same reporter construct such that SEAP activity is independent of the NS3 protease.

Protocol of the assay: Huh-7 cells, grown in CHO-SFMII (Life Technologies) + 10% FCS (fetal calf serum) were co-transfected with the two DNA constructs in the
20 following proportions:
7 µg NS3+ 500ng SEAP + 800µl FuGENE (Boehringer Mannheim) per 4 X 10⁶ Huh-7 cells. After 5 hours at 37°C, the cells were washed, trypsinized and plated (at 80 000 cells/well) in 96-well plates containing a range of concentrations of the compounds to be tested. After a 24-hour incubation period, the SEAP activity in the medium was
25 measured with the Phospha-Light kit (Tropix).

Analysis of the percent inhibition of SEAP activity with respect to compound concentration was performed with the SAS software to obtain the EC₅₀.

TABLES OF COMPOUNDS

The following tables list compounds representative of the invention.

All compounds listed in Tables 1 to 9 were found to be active in the enzymatic assay presented in Example 48. A number accompanied by an asterisk (*) represents

5 enzymatic activity obtained with the radiometric assay presented in Example 49 with IC_{50} 's under $50\mu M$. In these enzymatic assays, the following grading was used:

$A \geq 1\mu M$; $1\mu M > B > 0.1\mu M$; and $C \leq 0.1\mu M$.

Several compounds were tested in the specificity assays presented in Example 50 and were found to be specific for the NS3 protease. In general, the results from the

10 different specificity assays are the following: HLE $>300\mu M$; PPE $>300\mu M$; α -Chym. $>300\mu M$; Cat. B $>300\mu M$; indicating that these compounds are highly specific toward the HCV NS3 protease and are not expected to exhibit serious side-effects.

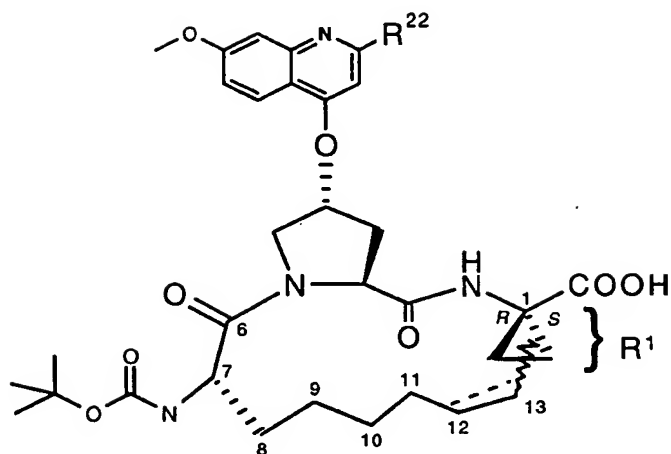
In addition, certain of these compounds were tested in the cell-based assay described in Example 51 and were found to have activity with an EC_{50} below $10\mu M$,

15 strongly indicating that these compounds can cross the cell membrane. Particularly, compounds of Tables 7, 8 and 9 have been assessed in the cellular assay and the result indicated in the last column. In this cellular assay, the following coding was used: $A > 1\mu M$; $B \leq 1\mu M$.

The following abbreviations are used within the following tables:

20 MS: Electrospray mass spectral data; m/z MH^+ except when otherwise indicated by an asterisk* = m/z MH^- ; Ac: acetyl; Bn: benzyl; Boc: *tert*-butoxycarbonyl; Ph: phenyl; Pr: propyl.

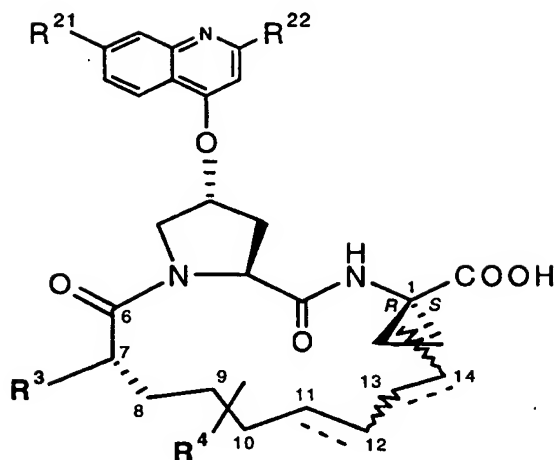
TABLE 1

single stereoisomer at R¹

Cpd. #	double bond	D-R ¹ bond stereochem	R ²²	MS	enzyme activity.
101	12,13- <i>trans</i>	1R, D <i>syn</i> to amide	phenyl	685.8	A*
102	none	1R, D <i>syn</i> to acid	phenyl	687.2	C
103	none	1R D <i>syn</i> to amide	phenyl	687.2	A*


111

TABLE 2

single stereoisomer at R¹

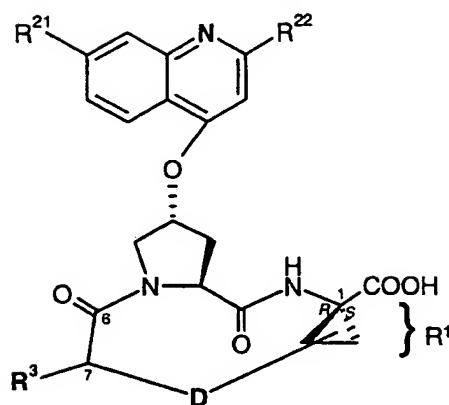
Cpd #	R ³	R ⁴	double bond	D-R ¹ bond stereochem	R ²¹	R ²²	MS	enz. act.
202	NH-Boc	H	11,12- <i>trans</i>	1R or 1S, D syn to acid	H	H	593.7	B
203	NH-acetyl	H	11,12- <i>trans</i>	1R or 1S, D syn to acid	H	H	535.6	A
205	NH-Boc	11-OH 12-OH cis	none	1R or 1S, D syn to acid	H	H	627.7	B
206	NH-Boc	H	13,14- <i>cis</i>	1R, D syn to acid	H	H	593.7	C
207	NH-Boc	H	13,14- <i>cis</i>	1R, D syn to acid	OMe	H	623.7	C
208	NH-Boc	H	13,14- <i>cis</i>	1R, D syn to acid	OMe	phenyl	699.8	C
209	NH-C(O)- NH- <i>t</i> Bu	H	13,14- <i>cis</i>	1R, D syn to acid	OMe	phenyl	698.8	C

112

Cpd #	R ³	R ⁴	double bond	D-R ¹ bond stereochem	R ²¹	R ²²	MS	enz. act.
210	NH-Boc	H	13,14- <i>cis</i>	1S, D syn to acid	OMe	phenyl	699.8	A*
211	NH ₂	H	13,14- <i>cis</i>	1R, D syn to acid	OMe	phenyl	599.7	C
213	OH (one isomer)	H	13,14- <i>cis</i>	1R, D syn to acid	OMe	H	524.6	B
214	NH-Boc	10-oxo	13,14- <i>cis</i>	1R, D syn to acid	OMe	phenyl	713.8	C
215	NH-Boc	H	none	1R, D syn to acid	OMe	phenyl	701.8	C
217	NH-Boc	10-OH (mixt dia stereo)	13,14- <i>cis</i>	1R, D syn to acid	OMe	phenyl	715.8	C
218	NH-Boc	10-oxo	13,14- <i>cis</i>	1R, D <i>syn</i> to amide	OMe	phenyl	713.8	C
219	NH-Ac	H	none	1R, D syn to acid	OMe	phenyl	643.2	C
220	NH-Boc	H	13,14- <i>cis</i>	1R, D <i>syn</i> to amide	OMe		706.2	C

113

TABLE 3

single stereoisomer at R¹

Cpd. #	R ³	- D -	D-R ¹ bond stereochem	R ²¹	R ²²	MS	enz act.
301	NH- Boc		1R or 1S, D syn to acid	H	H	621.7	B
302	NH- Boc		1R, D syn to amide	OMe	Ph	671	A
303	NH- Boc		1R, D syn to amide	OMe	Ph	701.3	B
304	NH- Boc		1R, D syn to acid	OMe	Ph	711.1	C
305	HO		1R, D syn to acid	OMe	Ph	602.2	B
306	NH- Boc		1R, D syn to amide	OMe	Ph	673.2	A*

114


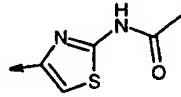
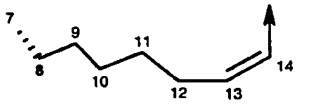
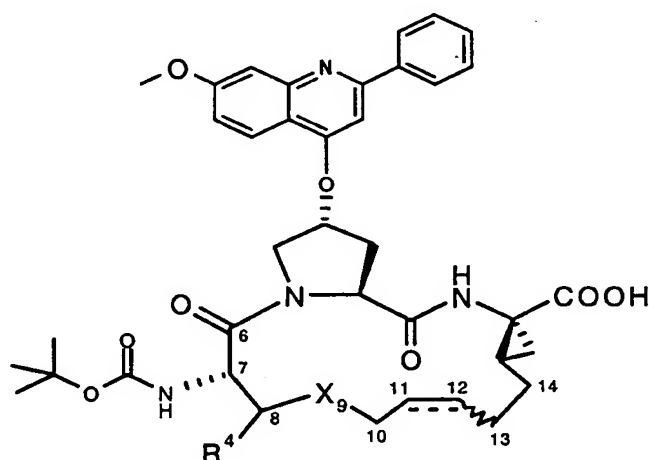







Cpd. #	R ³	- D -	D-R ¹ bond stereochem	R ²¹	R ²²	MS	enz act.
307	NH-Boc		1R, D <i>syn</i> to acid	OMe		777	C
308	NH-Ac		1R, D <i>syn</i> to acid	OMe	OEt	609.2	C

TABLE 4

D-R¹ bond *syn* to the acid

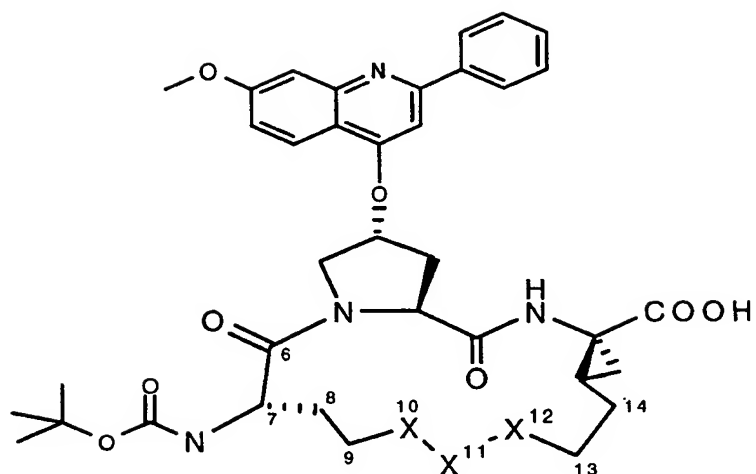
Cpd #	R ⁴	9-X	11, 12 double bond	MS	enzyme activity.
401	H	CH ₂	trans	699.3	C
402	H	CH ₂	cis	699.4	B
403	H	O	trans	701.3	C
404		O	trans	715.3	B

115

Cpd #	R ⁴	9-X	11, 12 double bond	MS	enzyme activity.
405	Me 	O	trans	715.2	C
406	H	O	none	703.3	C
407	Me 	O	none	717.3	B
408	Me 	O	none	717.3	C
409	Me 	O	cis	715.2	B
410	Me 	S	trans	731.3	C
411	Me 	S	cis	731.3	A*
412	8-(Me) ₂	9-S	cis	745.3	A

116

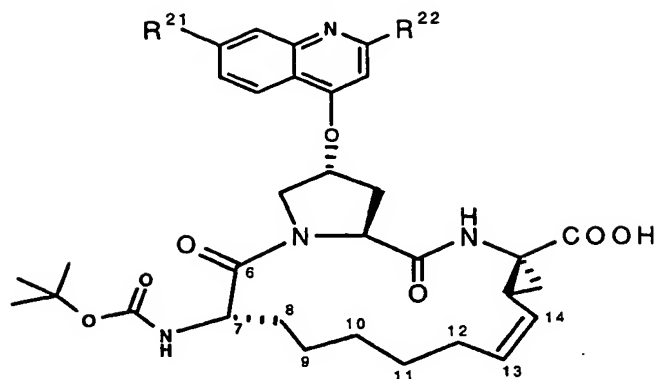
TABLE 5

D-R¹ bond *syn* to the acid

Cpd #	10-X	11-X	12-X	MS	enzyme activity.
501	CH ₂	O	CH ₂	703.2	C
502	CH ₂	CH ₂	CH ₂	701	C
503	CH ₂	CH ₂	NH	702.3	A
504	CH ₂	CH ₂	N(Me)	716.3	A*
505	CH ₂	CH ₂	N(CO)Me	744.3	B
506	CH ₂	CH ₂	N(CO)Ph	806.3	B
507	NH	CH ₂	CH ₂	702.3	C
508	N(CO)Me	CH ₂	CH ₂	744.3	C

117

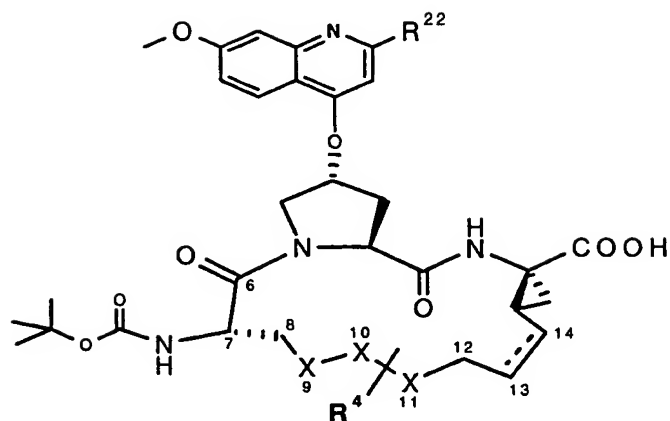
TABLE 6

D-R¹ bond *syn* to the acid

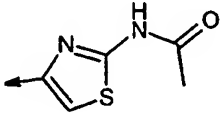
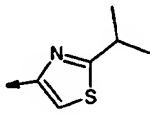
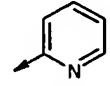
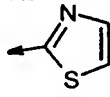
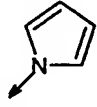
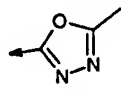
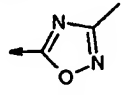
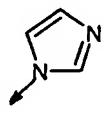
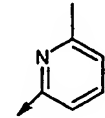
Cpd #	R ²¹	R ²²	MS	enzyme activity.
601	N(Me) ₂		776.2	C
602	OH	(CF ₃)	675.2*	C
603	OMe		690.1	C

118

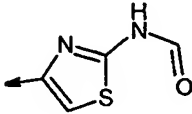
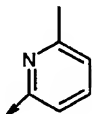
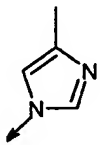
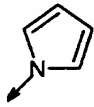
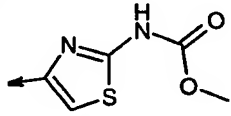
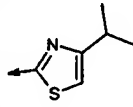

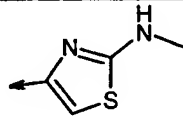
TABLE 7

D-R¹ bond *syn* to the acid

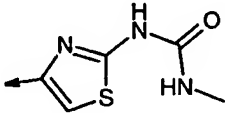
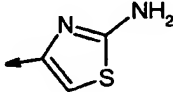

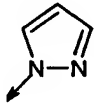
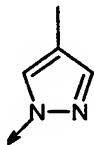
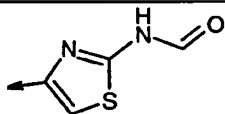
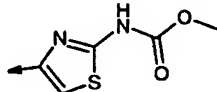
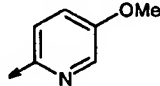
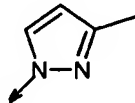
Cpd #	R ⁴	X ⁹ ; X ¹⁰ ; or X ¹¹	13,14 double bond	R ²²	MS	cell. act.
701	H	11-O	Cis	phenyl	701.3	A
702	H	CH ₂	Cis		763.1	B
703	H	CH ₂	None		751.4	B
704	H	CH ₂	Cis		700.3	B
705	H	CH ₂	Cis		706.2	B
707	H	CH ₂	Cis		748.2	B
708	H	CH ₂	Cis		749.2	B

Cpd #	R ⁴	X ⁹ ; X ¹⁰ ; or X ¹¹	13,14 double bond	R ²²	MS	cell. act.
709	H	CH ₂	None		765.2	B
710	H	CH ₂	None		750.1	B
711	H	CH ₂	None		702.2	B
712	H	CH ₂	Cis	-OEt	667.3	B
713	H	CH ₂	None		708.1	B
714	H	CH ₂	None	-OEt	669.3	B
715	H	CH ₂	Cis		688.3	B
716	H	CH ₂	Cis		705.3	A
717	H	CH ₂	Cis		705.2	B
718	H	CH ₂	Cis		689.3	B
719	H	CH ₂	Cis		714.2	B

120

Cpd #	R ⁴	X ⁹ ; X ¹⁰ ; or X ¹¹	13,14 double bond	R ²²	MS	cell. act.
720	H	CH ₂	None		751.2	B
721	H	CH ₂	None		716.3	B
722	H	CH ₂	Cis		703.3	B
723	H	CH ₂	None		690.3	B
724	H	CH ₂	None		781.1	B
725	H	CH ₂	Cis		748.2	B
726	H	CH ₂	Cis		703.2	B
727	H	CH ₂	Cis	-CH ₂ -OMe	667.3	A
728	H	CH ₂	Cis	Me	637.3	A
729	H	CH ₂	Cis		735.2	B

121

Cpd #	R ⁴	X ⁹ ; X ¹⁰ ; or X ¹¹	13,14 double bond	R ²²	MS	cell. act.
730	H	CH ₂	None		780.1	B
731	H	CH ₂	Cis		721.1	B
732	H	CH ₂	Cis		705.3	A
733	H	CH ₂	Cis		689.3	B
734	H	CH ₂	Cis		703.2	B
735	H	CH ₂	Cis		749.2	B
736	H	CH ₂	Cis		779.2	B
737	H	CH ₂	Cis		730.2	B
738	H	CH ₂	Cis		703.2	B
739	10-(R) Me	CH ₂	None	Ph	715.2	B
740	10-(S) Me	CH ₂	none	Ph	715.3	B

122

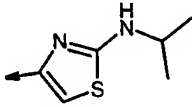
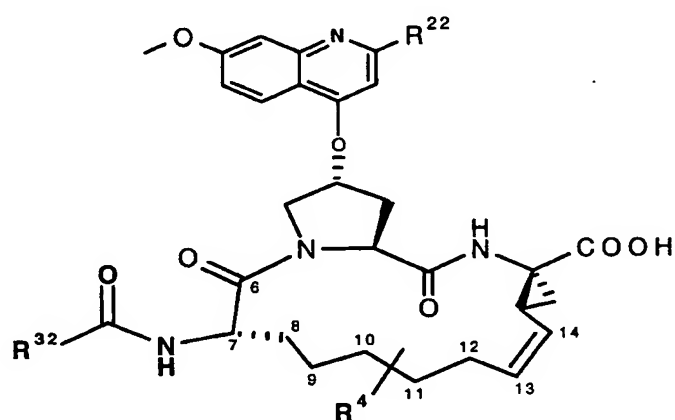
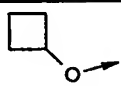
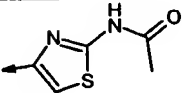
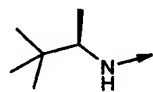
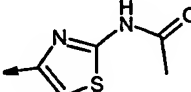
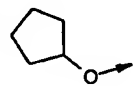
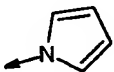
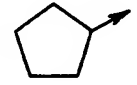
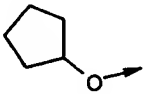
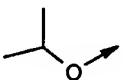
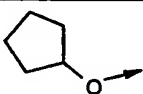
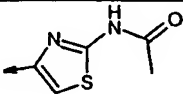
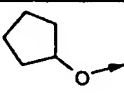
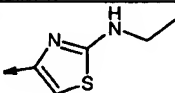
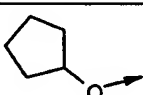
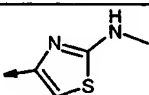
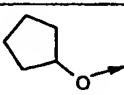
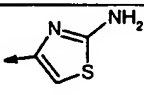

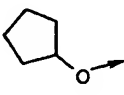
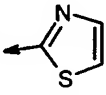
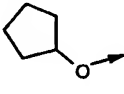
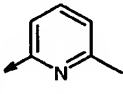
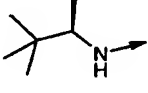
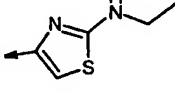
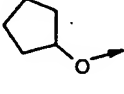
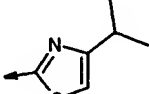
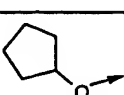
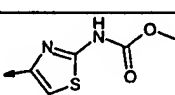
Cpd #	R ⁴	X ⁹ ; X ¹⁰ ; or X ¹¹	13,14 double bond	R ²²	MS	cell. act.
741	H	CH ₂	Cis		763.1	B

TABLE 8

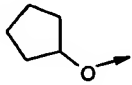
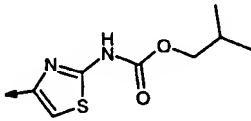
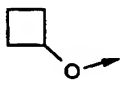
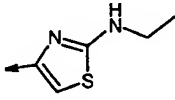
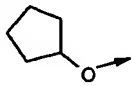

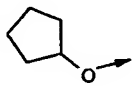
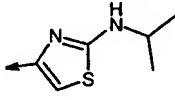
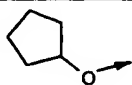

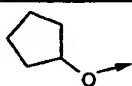
D-R¹ bond *syn* to the acid, double bond 13,14: *cis*

Cpd #	R ³²	R ⁴	R ²²	MS	cell. act.
801		H		761.2	B
803	<i>n</i> -Pr	H	OEt	637.3	A
804		H		790.3	B
805		H		700.1	B
806		H	OEt	663.2	A

123

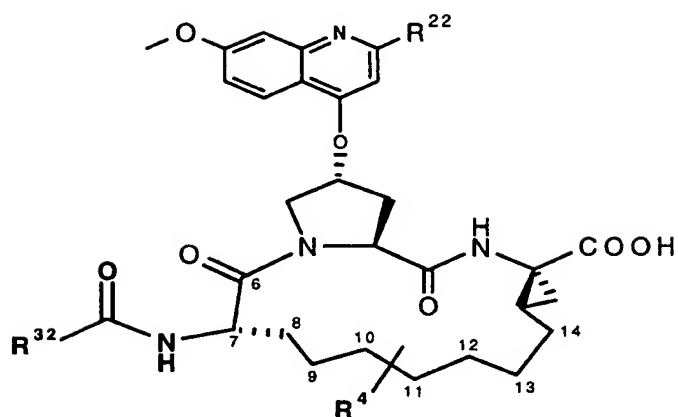
Cpd #	R ³²	R ⁴	R ²²	MS	cell. act.
807		H	OEt	679.3	B
808		H	OEt	653.2	A
809		H		775.1	B
810		H		761.2	B
811		H		747.2	B
812		H		733.2	B
813		H	OEt	691.3	B
814		H		718.3	B
815		H		726.3	B
816		H		776.3	B
817		H		760.2	B
818		H		791.1	B

124

Cpd #	R ³²	R ⁴	R ²²	MS	cell. act.
819		H		833.2	B
820		H		747.2	B
821		H		700.9	B
822		H		775.4	B
823		H		715.2	B
824		10- (R) Me	OEt	693.0	B

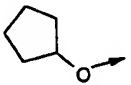
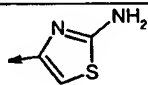
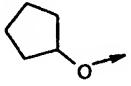
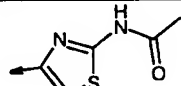
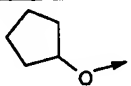
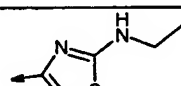
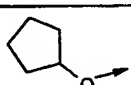
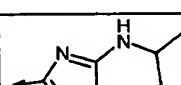
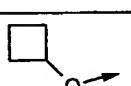
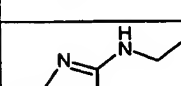
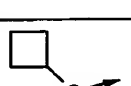
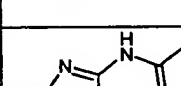
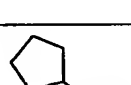
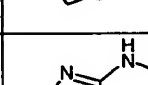
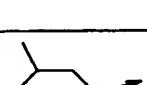
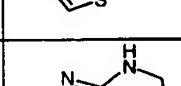

125

TABLE 9

D-R¹ bond *syn* to the acid

Cpd #	R ³²	R ⁴	R ²²	MS	cell. act.
901		H	OEt	681.3	B
902		H		719.9	B
903		H		705.9	B
904		H		703.0	B
905		H		689.0	B
906		H		762.0	B
907		H		702.0	B

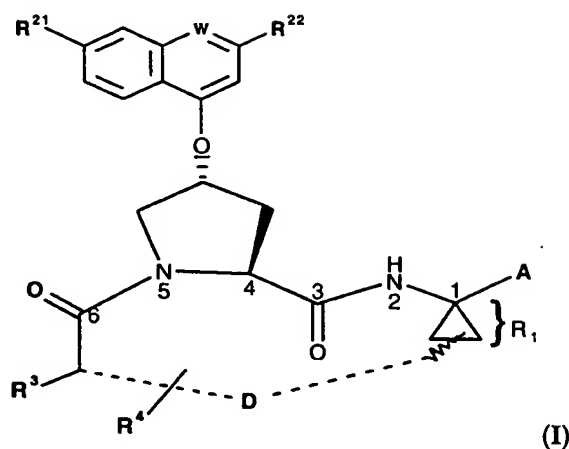
126

Cpd #	R ³²	R ⁴	R ²²	MS	cell. act.
908		H		735.2	B
909		H		777.0	B
910		H		763.1	B
911		H		777.0	B
912		H		748.9	B
913		H		762.9	B
914		H		749.0	B
915		H		751.1	B
916		10 (R) Me	OEt	695.2	B

CLAIMS

What is claimed is:

1. A compound of formula (I):



- 5 wherein W is CH or N ,
 R^{21} is H , halo, C_{1-6} alkyl, C_{3-6} cycloalkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{3-6} cycloalkoxy, hydroxy, or $N(R^{23})_2$,
 wherein each R^{23} is independently H , C_{1-6} alkyl or C_{3-6} cycloalkyl;
 R^{22} is H , halo, C_{1-6} alkyl, C_{3-6} cycloalkyl, C_{1-6} haloalkyl, C_{1-6} thioalkyl, C_{1-6} alkoxy, C_{3-6} cycloalkoxy, C_{2-7} alkoxyalkyl, C_{3-6} cycloalkyl, $C_{6 \text{ or } 10}$ aryl or Het, wherein Het is a five-, six-, or seven-membered saturated or unsaturated heterocycle containing from one to four heteroatoms selected from nitrogen, oxygen and sulfur;
 said cycloalkyl, aryl or Het being substituted with R^{24} ,
 wherein R^{24} is H , halo, C_{1-6} alkyl, C_{3-6} cycloalkyl, C_{1-6} alkoxy, C_{3-6} cycloalkoxy, NO_2 ,
 15 $N(R^{25})_2$, $NH-C(O)-R^{25}$; or $NH-C(O)-NH-R^{25}$, wherein each R^{25} is independently: H , C_{1-6} alkyl or C_{3-6} cycloalkyl;
 or R^{24} is $NH-C(O)-OR^{26}$ wherein R^{26} is C_{1-6} alkyl or C_{3-6} cycloalkyl;
 R^3 is hydroxy, NH_2 , or a group of formula $-NH-R^{31}$, wherein R^{31} is $C_{6 \text{ or } 10}$ aryl, heteroaryl, $-C(O)-R^{32}$, $-C(O)-NHR^{32}$ or $-C(O)-OR^{32}$,

wherein R^{32} is C_{1-6} alkyl or C_{3-6} cycloalkyl;

D is a 5 to 10-atom saturated or unsaturated alkylene chain optionally containing one to three heteroatoms independently selected from: O, S, or $N-R^{41}$, wherein

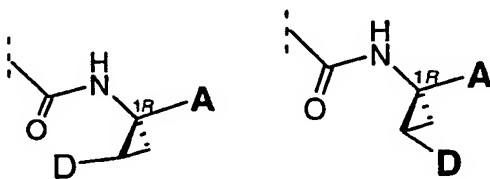
R^{41} is H, C_{1-6} alkyl, C_{3-6} cycloalkyl or $-C(O)-R^{42}$, wherein R^{42} is C_{1-6} alkyl, C_{3-6}

5 cycloalkyl or $C_{6 \text{ or } 10}$ aryl;

R^4 is H or from one to three substituents at any carbon atom of said chain D, said substituent independently selected from the group consisting of: C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, hydroxy, halo, amino, oxo, thio or C_{1-6} thioalkyl, and

10 A is an amide of formula $-C(O)-NH-R^5$, wherein R^5 is selected from the group consisting of: C_{1-8} alkyl, C_{3-6} cycloalkyl, $C_{6 \text{ or } 10}$ aryl or C_{7-16} aralkyl; or A is a carboxylic acid or a pharmaceutically acceptable salt or ester thereof.

2. A compound of formula I according to claim 1, wherein said R^1 moiety is
15 selected from the 2 different diastereoisomers represented by structures (i) and (ii):



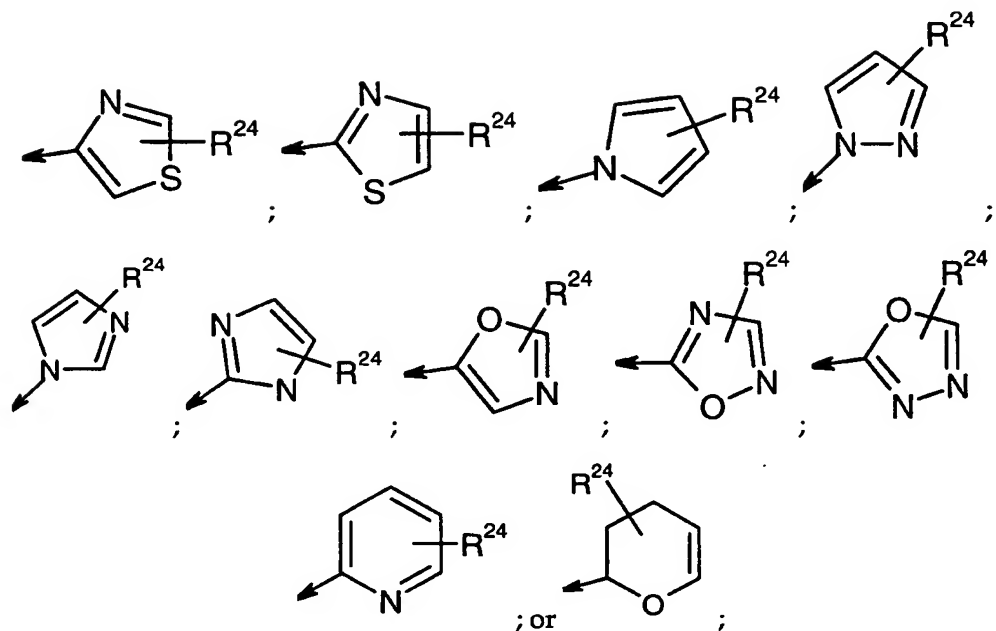
D syn to the amide (i), or D syn to A (ii).

3. The compound of formula I according to claim 2, wherein D is linked *syn* to
20 A as represented by formula (ii).

4. The compound of formula I according to claim 1, wherein W is N;

R^{21} is H, C_{1-6} alkyl, C_{1-6} alkoxy, hydroxy, chloro, or $N(R^{23})_2$, wherein R^{23} is H or C_{1-6} alkyl;

25 R^{22} is H, C_{1-6} thioalkyl, C_{1-6} alkoxy, phenyl or Het selected from the group consisting of:



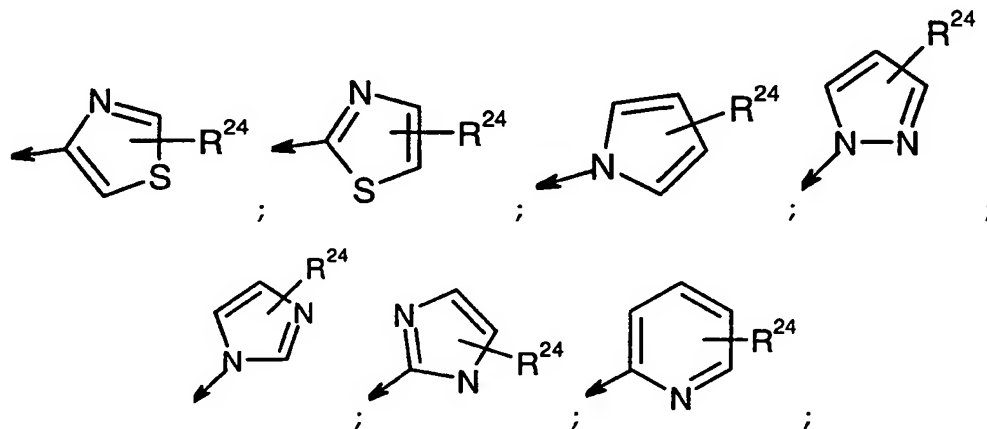
wherein R^{24} is H, C_{1-6} alkyl, $NH-R^{25}$, $NH-C(O)-R^{25}$, $NH-C(O)-NH-R^{25}$,

- 5 wherein each R^{25} is independently: H, C_{1-6} alkyl, or C_{3-6} cycloalkyl;
or $NH-C(O)-OR^{26}$, wherein R^{26} is C_{1-6} alkyl.

5. A compound of formula I according to claim 4, wherein R^{21} is H or C_{1-6} alkoxy.

10

6. The compound of formula I according to claim 4, wherein R^{22} is C_{1-4} alkoxy, phenyl or Het selected from the group consisting of:

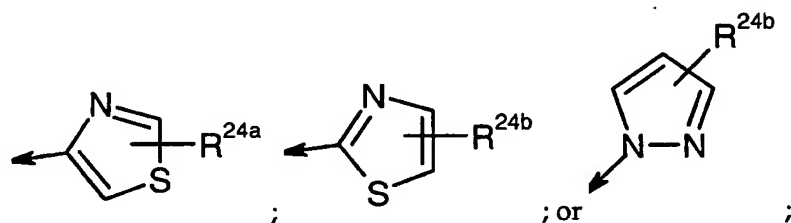


wherein R^{24} is H, C_{1-6} alkyl, $NH-R^{25}$, or $NH-C(O)-R^{25}$;

wherein each R^{25} is C_{1-6} alkyl or C_{3-6} cycloalkyl,
or $NH-C(O)-OR^{26}$, wherein R^{26} is as defined in claim 4.

5 7. A compound of formula I according to claim 6, wherein R^{21} is methoxy.

8. The compound of formula I according to claim 7, wherein R^{22} is ethoxy, or
Het selected from the group consisting of:



10 wherein R^{24a} is $NH-R^{25}$ or $NH-C(O)-R^{25}$, wherein R^{25} is C_{1-6} alkyl;
or R^{24a} is $NH-C(O)-OR^{26}$, wherein R^{26} is C_{1-6} alkyl, and R^{24b} is H or C_{1-6} alkyl.

9. The compound of formula I according to claim 1, wherein R^3 is an amide of
formula $NH-C(O)R^{32}$ or a urea of formula $NH-C(O)-NH-R^{32}$ or a carbamate of
15 formula $NH-C(O)-OR^{32}$, wherein R^{32} is C_{1-6} alkyl, or C_{3-6} cycloalkyl.

10. The compound of formula I according to claim 9, wherein R^3 is a urea or a
carbamate, wherein R^{32} is C_{1-6} alkyl or C_{4-6} cycloalkyl.

20 11. The compound of formula I according to claim 10, wherein R^3 is a carbamate
and R^{32} is *tert*-butyl, cyclobutyl or cyclopentyl.

12. The compound of formula I according to claim 1, wherein D is a 6 to 8 atom
saturated or unsaturated alkylene chain optionally containing one or two
25 heteroatoms independently selected from: O, S or $N-R^{41}$, wherein R^{41} is H, C_{1-6} alkyl,

or C₂₋₇ acyl.

13. The compound of formula I according to claim 12, wherein D optionally contains one heteroatom selected from: NH, or N-C₂₋₇ acyl.

5

14. The compound according to claim 13, wherein said heteroatom is selected from: NH or N(Ac).

15. The compound according to claim 13, wherein said D chain contains 7 atoms.

10

16. The compound according to claim 15, wherein said heteroatom is at position 10 of said D chain.

17. The compound according to claim 13, wherein said D chain is saturated.

15

18. The compound of formula I according to claim 12, wherein D is a 6 to 8 atom saturated or unsaturated alkylene chain optionally containing one heteroatom selected from: O, or S.

20 19. The compound according to claim 18, wherein said D chain contains 7 atoms.

20. The compound according to claim 19, wherein said heteroatom is at position 9 of said D chain.

25 21. The compound according to claim 20, wherein said D chain is substituted at position 8 with R⁴, wherein R⁴ is H or C₁₋₆ alkyl.

22. The compound according to claim 21, wherein said R⁴ is H or methyl.

30 23. The compound according to claim 22, wherein said R⁴ is H or 8-(S)-Me.

132

24. The compound according to claim 23, wherein said **D** chain is saturated.
25. The compound according to claim 19, wherein said **D** chain contains one double bond at position 11,12.
- 5 26. The compound according to claim 25, wherein said double bond is *trans*.
27. The compound of formula I according to claim 12, wherein **D** is a 6 to 8 atom, saturated or unsaturated, all carbon alkylene chain.
- 10 28. The compound of formula I according to claim 27, wherein **D** is a 7 atom chain.
29. The compound of formula I according to claim 28, wherein **D** is saturated.
- 15 30. The compound according to claim 29, wherein said **D** chain is substituted with R^4 , wherein R^4 is H, oxo, hydroxy, alkoxy or alkyl.
31. The compound according to claim 30, wherein said R^4 is H or C_{1-6} alkyl.
- 20 32. The compound according to claim 31, wherein said R^4 is H or methyl.
33. The compound according to claim 32, wherein said R^4 is H or 10-(*S*)-Me.
- 25 34. The compound of formula I according to claim 28, wherein **D** contains one double bond.
35. The compound of formula I according to claim 34, wherein said double bond is at position 13,14 of said **D** chain.
- 30 36. The compound of formula I according to claim 35, wherein said double bond

is *cis*.

37. The compound according to claim 36, wherein said D chain is substituted with R^4 , wherein R^4 is H, oxo, hydroxy, C_{1-6} alkoxy or C_{1-6} alkyl.

5

38. The compound according to claim 37, wherein said R^4 is H or C_{1-6} alkyl.

39. The compound according to claim 38, wherein said R^4 is H or methyl.

10 40. The compound according to claim 39, wherein said R^4 is H or 10-(S)-Me.

41. The compound of formula I according to claim 1, wherein A is a carboxylic acid.

15 42. A compound according to claim 1, wherein W is N;

R^3 is a group of formula or $-NH-C(O)-NHR^{32}$ or $-NH-C(O)-OR^{32}$,

wherein R^{32} is C_{1-4} alkyl or C_{4-6} cycloalkyl;

D is a 6 to 8 atom saturated or unsaturated alkylene chain linked to R^1 *syn* to A, optionally containing one or two heteroatoms independently selected from: O, S or

20 $N-R^{41}$, wherein R^{41} is H, or C_{2-7} acyl;

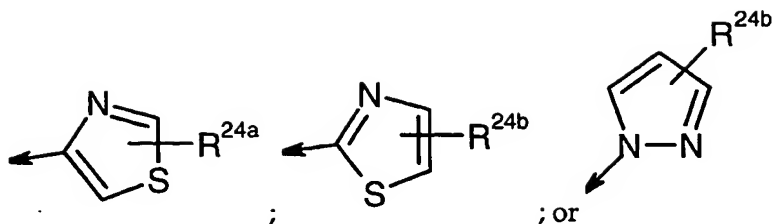
R^4 is H, or from one to three substituents independently selected from hydroxy, or C_{1-6} alkyl; and

A is a carboxylic acid, or a pharmaceutically acceptable salt or ester thereof.

25 43. The compound of formula I according to claim 42, wherein R^{21} is H or methoxy;

R^{22} is C_{1-6} alkoxy, or Het selected from the group consisting of:

134



wherein R^{24a} is H, C_{1-6} alkyl, $NH-R^{25}$, $NH-C(O)-R^{25}$; or $NH-C(O)-NH-R^{25}$,

wherein R^{25} is: H, C_{1-6} alkyl or C_{3-6} cycloalkyl;

or R^{24a} is $NH-C(O)-OR^{26}$, wherein R^{26} is C_{1-6} alkyl or C_{3-6} cycloalkyl;

5 and R^{24b} is H or C_{1-6} alkyl;

R^3 is urea of the formula $N-C(O)-NHR^{32}$ or a carbamate of the formula $N-C(O)-OR^{32}$,

wherein R^{32} is C_{1-6} alkyl or C_{3-6} cycloalkyl;

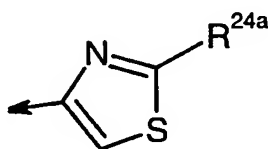
D is a 7-atom alkylene chain optionally containing one double bond at position 11,12 or 13,14;

10 said D chain optionally containing one heteroatom independently selected from: O, S, NH, N(Me), or N(Ac); and

R^4 is H or C_{1-6} alkyl.

44. The compound of formula I according to claim 43, wherein R^{21} is methoxy,

15 and R^{22} is ethoxy or



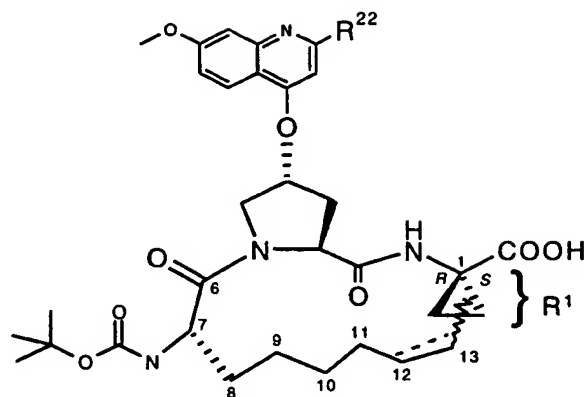
wherein R^{24a} is $NH-(C_{1-4}$ alkyl); $NH-C(O)-(C_{1-4}$ alkyl); $NH-C(O)-O-(C_{1-4}$ alkyl); or $NH-C(O)-NH-(C_{1-4}$ alkyl);

D is a C7 all carbon chain, saturated or containing one *cis* double bond at position

20 13,14.

135

45. A compound of formula:

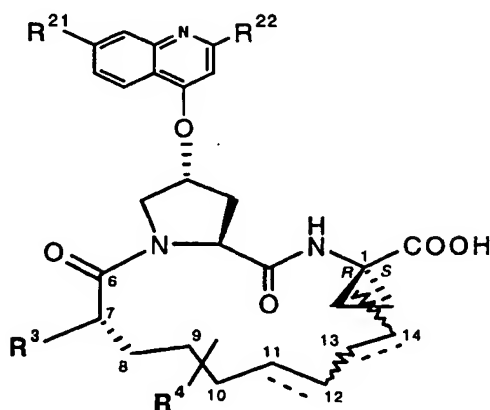


comprising a single stereoisomer at R^1 , wherein said double bond, D- R^1 bond stereochemistry and R^{22} are defined as follows:

Cpd. #	double bond:	D- R^1 bond stereochem:	R^{22} :
101	12,13- <i>trans</i>	1R, D <i>syn</i> to amide	phenyl;
102	none	1R, D <i>syn</i> to acid	phenyl;
and			
103	none	1R D <i>syn</i> to amide	phenyl.

5

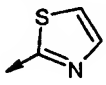
46. A compound of formula:



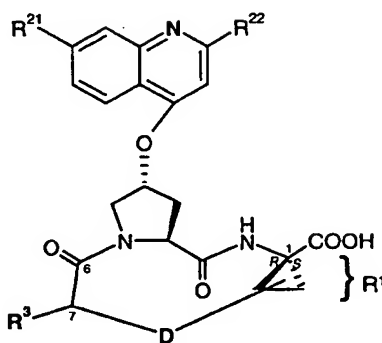
comprising a single stereoisomer at R^1 , wherein R^3 , R^4 , said double bond position, D- R^1 bond stereochemistry, R^{21} and R^{22} are defined as follows:


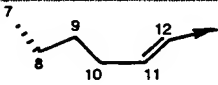
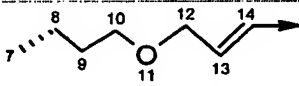
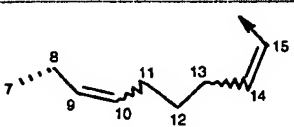
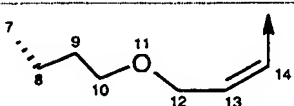
Cpd #	R ³ :	R ⁴ :	double bond:	D-R ¹ bond stereochemistry:	R ²¹ :	R ²² :
202	NH-Boc	H	11,12- <i>trans</i>	1R or 1S, D syn to acid	H	H;
203	NH-acetyl	H	11,12- <i>trans</i>	1R or 1S, D syn to acid	H	H;
205	NH-Boc	11-OH 12-OH <i>cis</i>	none	1R or 1S, D syn to acid	H	H;
206	NH-Boc	H	13,14- <i>cis</i>	1R, D syn to acid	H	H;
207	NH-Boc	H	13,14- <i>cis</i>	1R, D syn to acid	OMe	H;
208	NH-Boc	H	13,14- <i>cis</i>	1R, D syn to acid	OMe	phenyl;
209	NH-C(O)- NH- <i>t</i> Bu	H	13,14- <i>cis</i>	1R, D syn to acid	OMe	phenyl;
210	NH-Boc	H	13,14- <i>cis</i>	1S, D syn to acid	OMe	phenyl;
211	NH ₂	H	13,14- <i>cis</i>	1R, D syn to acid	OMe	phenyl;
213	OH (one isomer)	H	13,14- <i>cis</i>	1R, D syn to acid	OMe	H;
214	NH-Boc	10-oxo	13,14- <i>cis</i>	1R, D syn to acid	OMe	phenyl;
215	NH-Boc	H	none	1R, D syn to acid	OMe	phenyl;
217	NH-Boc	10-OH (mixt dia stereo)	13,14- <i>cis</i>	1R, D syn to acid	OMe	phenyl;
218	NH-Boc	10-oxo	13,14- <i>cis</i>	1R, D syn to amide	OMe	phenyl;
219	NH-Ac	H	none	1R, D syn to acid	OMe	phenyl;

137

Cpd #	R ³ :	R ⁴ :	double bond:	D-R ¹ bond stereochemistry:	R ²¹ :	R ²² :
and 220	NH-Boc	H	13,14-cis	1R, D <i>syn</i> to amide	OMe	

47. A compound of formula:

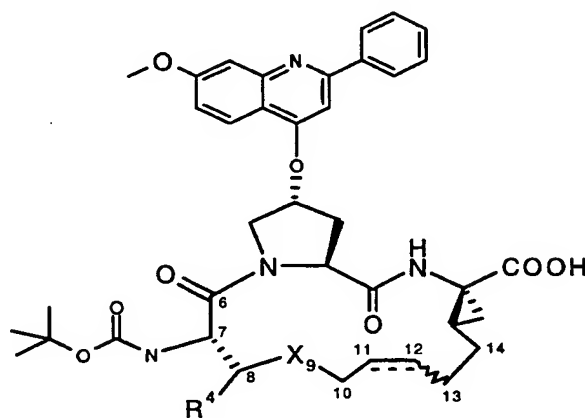
comprising a single stereoisomer at R¹, wherein R³, D, D-R¹ bond stereochemistry,5 R²¹ and R²² are defined as follows:

Cpd. #	R ³ :	- D -:	D-R ¹ bond stereochemistry:	R ²¹ :	R ²² :
301	NH-Boc		1R or 1S, D <i>syn</i> to acid	H	H;
302	NH-Boc		1R, D <i>syn</i> to amide	OMe	Ph;
303	NH-Boc		1R, D <i>syn</i> to amide	OMe	Ph;
304	NH-Boc		1R, D <i>syn</i> to acid	OMe	Ph;
305	HO		1R, D <i>syn</i> to acid	OMe	Ph;

138

Cpd. #	R ³ :	- D -:	D-R ¹ bond stereochemistry:	R ²¹ :	R ²² :
306	NH-Boc		1R, D <i>syn</i> to amide	OMe	Ph;
307	NH-Boc		1R, D <i>syn</i> to acid	OMe	
and 308	NH-Ac		1R, D <i>syn</i> to acid	OMe	OEt.







48. A compound of formula:



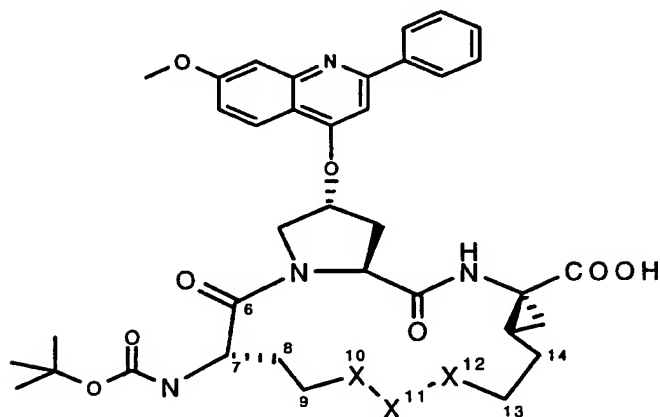
wherein said D-R¹ bond is *syn* to acid, R⁴, X₉, and said 11,12 double bond are defined as follows:

Cpd #	R ⁴ :	X ₉ :	11, 12 double bond:
401	H	CH ₂	trans;
402	H	CH ₂	cis;
403	H	O	trans;
404		O	trans;

139

Cpd #	R ⁴ :	X ₉ :	11, 12 double bond:
405	Me 	O	trans;
406	H	O	none;
407	Me 	O	none;
408	Me 	O	none;
409	Me 	O	cis;
410	Me 	S	trans;
411	Me 	S	cis;
and 412	8-(Me) ₂	9-S	cis.

49. A compound of formula:

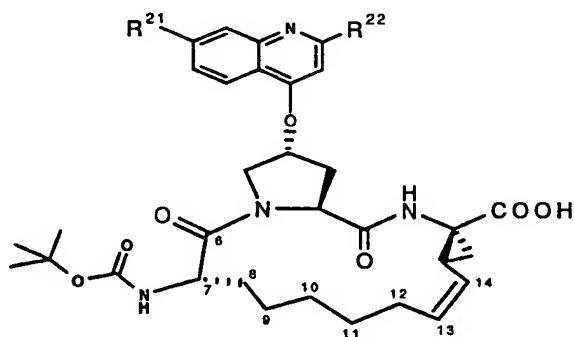


wherein said D-R¹ bond is *syn* to acid, X₁₀, X₁₁, and X₁₂ are defined as follows:

140

Cpd #	X ₁₀ :	X ₁₁ :	X ₁₂ :
501	CH ₂	O	CH ₂ ;
502	CH ₂	CH ₂	CH ₂ ;
503	CH ₂	CH ₂	NH;
504	CH ₂	CH ₂	N(Me);
505	CH ₂	CH ₂	N(CO)Me;
506	CH ₂	CH ₂	N(CO)Ph;
507	NH	CH ₂	CH ₂ ;
and 508	N(CO)Me	CH ₂	CH ₂ .

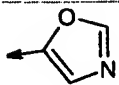
50. A compound of formula:



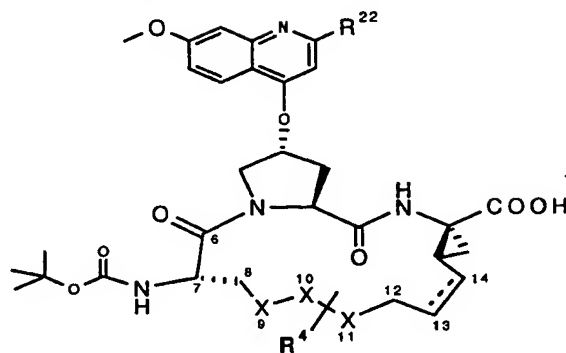
wherein said D-R¹ bond is *syn* to acid, R²¹ and R²² are defined as follows:

Cpd #	R ²¹ :	R ²² :
601	N(Me) ₂	
602	OH	(CF ₃);

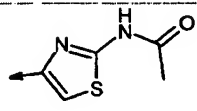
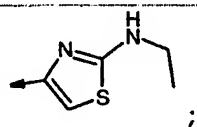
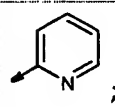
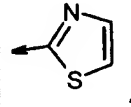
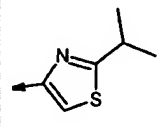
141

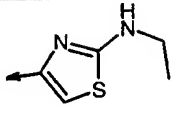
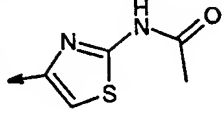
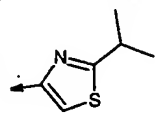
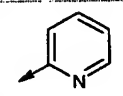
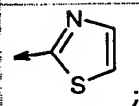
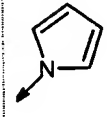
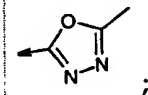
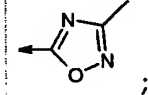
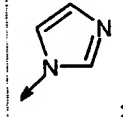
Cpd #	R ²¹ :	R ²² :
and 603	OMe	

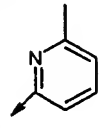
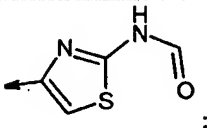
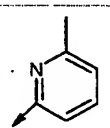
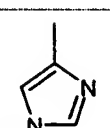
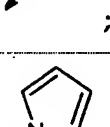
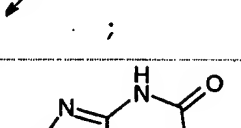
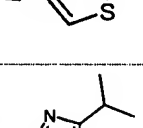
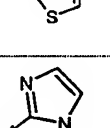
51. A compound of formula:

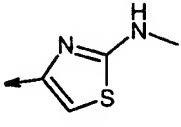
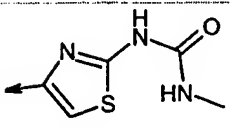
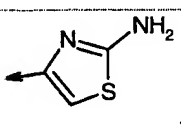
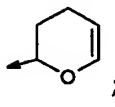
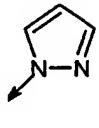
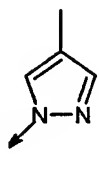
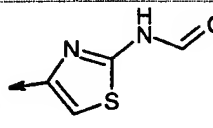
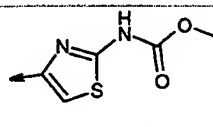
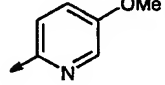
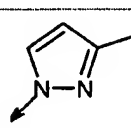


wherein said D-R¹ bond is *syn* to acid, R⁴, X₉, X₁₀, X₁₁, said 13,14 double bond and R²² are defined as follows:

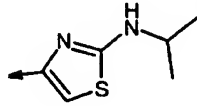
Cpd #	R ⁴ :	X ₉ ; X ₁₀ ; or X ₁₁ :	13,14 double bond:	R ²² :
701	H	11-O	Cis	phenyl;
702	H	CH ₂	Cis	 ;
703	H	CH ₂	None	 ;
704	H	CH ₂	Cis	 ;
705	H	CH ₂	Cis	 ;
707	H	CH ₂	Cis	 ;

Cpd #	R ⁴ :	X ₉ ; X ₁₀ or X ₁₁ :	13,14 double bond:	R ²² :
708	H	CH ₂	Cis	 ;
709	H	CH ₂	None	 ;
710	H	CH ₂	None	 ;
711	H	CH ₂	None	 ;
712	H	CH ₂	Cis	-OEt;
713	H	CH ₂	None	 ;
714	H	CH ₂	None	-OEt;
715	H	CH ₂	Cis	 ;
716	H	CH ₂	Cis	 ;
717	H	CH ₂	Cis	 ;
718	H	CH ₂	Cis	 ;

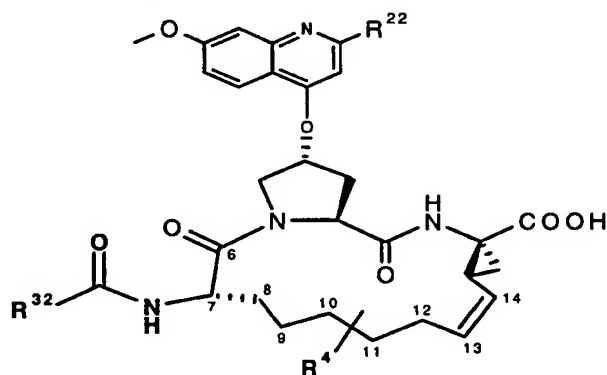
Cpd #	R ⁴ :	X ₉ ; X ₁₀ ⁱ or X ₁₁ :	13,14 double bond:	R ²² :
719	H	CH ₂	Cis	 ;
720	H	CH ₂	None	 ;
721	H	CH ₂	None	 ;
722	H	CH ₂	Cis	 ;
723	H	CH ₂	None	 ;
724	H	CH ₂	None	 ;
725	H	CH ₂	Cis	 ;
726	H	CH ₂	Cis	 ;
727	H	CH ₂	Cis	-CH ₂ -OMe;
728	H	CH ₂	Cis	Me;

Cpd #	R ⁴ :	X ₉ ; X ₁₀ or X ₁₁ :	13,14 double bond:	R ²² :
729	H	CH ₂	Cis	 ;
730	H	CH ₂	None	 ;
731	H	CH ₂	Cis	 ;
732	H	CH ₂	Cis	 ;
733	H	CH ₂	Cis	 ;
734	H	CH ₂	Cis	 ;
735	H	CH ₂	Cis	 ;
736	H	CH ₂	Cis	 ;
737	H	CH ₂	Cis	 ;
738	H	CH ₂	Cis	 ;


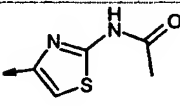
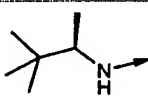
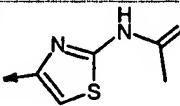
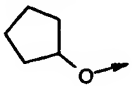
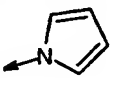
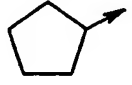
145

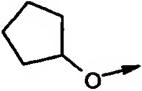
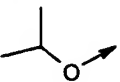
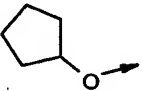
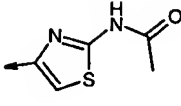
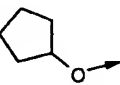
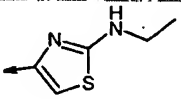
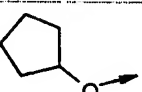
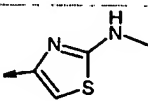
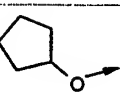
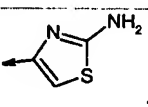

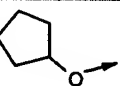
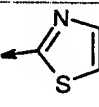
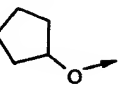
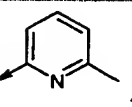
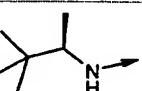
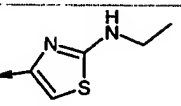
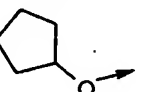
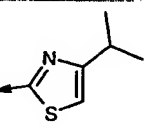
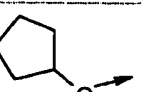
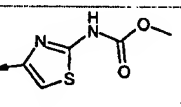
Cpd #	R ⁴ :	X ₉ ; X ₁₀ or X ₁₁ :	13,14 double bond:	R ²² :
739	10-(R) Me	CH ₂	none	Ph;
740	10-(S) Me	CH ₂	none	Ph;
and 741	H	CH ₂	Cis	

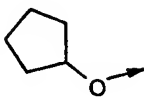
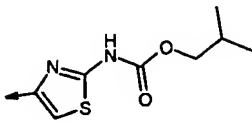
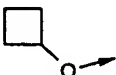
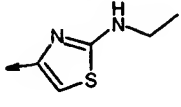
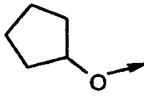

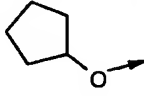
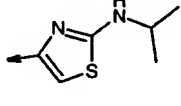
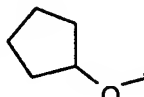
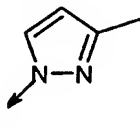
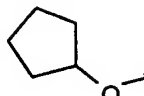
52. A compound of formula:



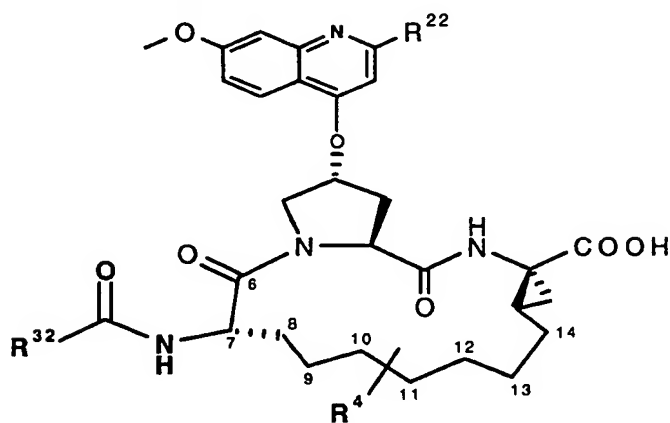
wherein said D-R¹ bond is *syn* to acid, said 13,14 double bond is *cis*, R³², R⁴ and R²² are defined as follows:

Cpd #	R ³² :	R ⁴ :	R ²² :
801		H	 ;
803	<i>n</i> -Pr	H	OEt;
804		H	 ;
805		H	 ;
806		H	OEt;

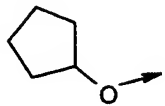
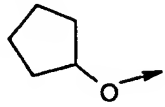
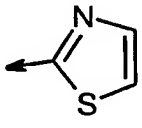
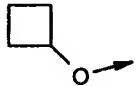
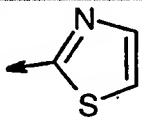
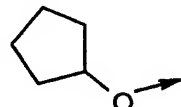
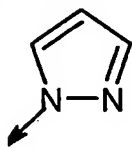
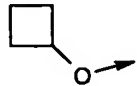
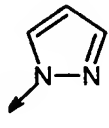
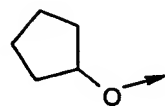
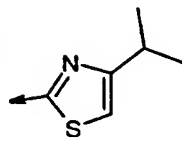
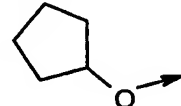
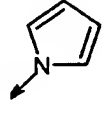
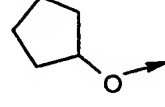
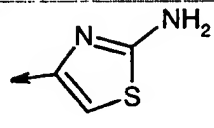
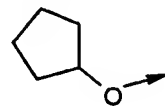
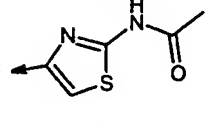
Cpd #	R ³² :	R ⁴ :	R ²² :
807		H	OEt;
808		H	OEt;
809		H	 ;
810		H	 ;
811		H	 ;
812		H	 ;
813		H	OEt;
814		H	 ;
815		H	 ;
816		H	 ;
817		H	 ;
818		H	 ;

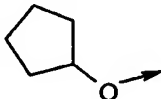
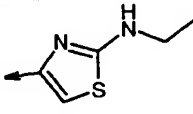
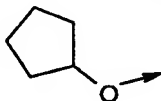
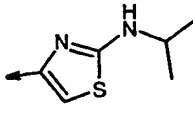
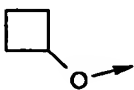
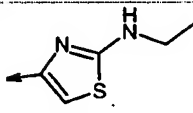
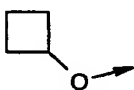
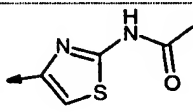
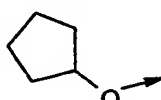
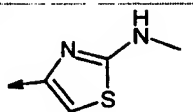
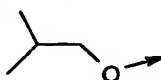
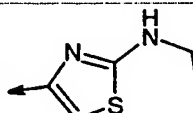
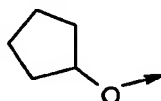
Cpd #	R ³² :	R ⁴ :	R ²² :
819		H	 ;
820		H	 ;
821		H	 ;
822		H	 ;
823		H	 ;
and 824		10- (R) Me	OEt.

53. A compound of formula:



wherein said D-R¹ bond *syn* to acid, R³², R⁴ and R²² are defined as follows:

Cpd #	R ³² :	R ⁴ :	R ²² :
901		H	OEt;
902		H	 ;
903		H	 ;
904		H	 ;
905		H	 ;
906		H	 ;
907		H	 ;
908		H	 ;
909		H	 ;

Cpd #	R ³² :	R ⁴ :	R ²² :
910		H	 ;
911		H	 ;
912		H	 ;
913		H	 ;
914		H	 ;
915		H	 ;
and 916		10 (R) Me	OEt.

54. A method of inhibiting the replication of hepatitis C virus by exposing the virus to a hepatitis C viral NS3 protease inhibiting amount of the compound of formula I according to claim 1.

5

55. A pharmaceutical composition comprising an anti-hepatitis C virally effective amount of a compound of formula I according to claim 1, or a therapeutically acceptable salt or ester thereof, in admixture with a pharmaceutically acceptable carrier medium or auxiliary agent.

10

56. The pharmaceutical composition according to claim 55, further comprising

an additional immunomodulatory agent.

57. The pharmaceutical composition according to claim 56, wherein said additional immunomodulatory agents is selected from the group consisting of: α -, β -
5 , and δ -interferons.

58. The pharmaceutical composition according to claim 55, further comprising an antiviral agent.

10 59. The pharmaceutical composition according to claim 58, wherein said antiviral agent is selected from the group consisting of: ribavirin and amantadine.

60. The pharmaceutical composition according to claim 55, further comprising another inhibitor of HCV protease.
15

61. The pharmaceutical composition according to claim 55, further comprising an inhibitor of other targets in the HCV life cycle, such as helicase, polymerase, or metalloprotease.

20 62. Use of pharmaceutical composition according to claim 55, for the manufacture of a medicament for treating a hepatitis C viral infection in a mammal.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 00/00353

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K5/08 C07K5/078 A61K38/05 A61K38/06 A61P31/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 99 07733 A (BOEHRINGER INGELHEIM CA LTD ;RANCOURT JEAN (CA); SIMONEAU BRUNO (C) 18 February 1999 (1999-02-18) cited in the application the whole document -----	1-62



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

16 June 2000

Date of mailing of the international search report

26/06/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.
Fax: (+31-70) 340-3016

Authorized officer

Groenendijk, M

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/CA 00/00353

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9907733 A	18-02-1999	AU 8795698 A EP 1003775 A	01-03-1999 31-05-2000
<hr/>			